Exhibit 63

Int. J. Cancer: **121,** 2364 2372 (2007)

Beyond foreign body induced carcinogenesis: Impact of reactive oxygen species derived from inflammatory cells in tumorigenic conversion and tumor progression

Futoshi Okada*

Department of Biomolecular Function, Graduate School of Medical Science, Yamagata University, Yamagata, Japan

Foreign body induced carcinogenesis is a traditional, maybe old, way of understanding cancer development. A number of novel approaches are available today to elucidate cancer development. However, there are things we learn from the old, and thus I bring out some examples of various clinical cases and experimental mod els of foreign body induced tumorigenesis. What is notable is that the foreign bodies themselves are unrelated to each other, whereas commonly underlying in them is to induce inflammatory reaction, especially stromal proliferation, where those exogenous materials are incorporated and undigested. Such foreign body induced car cinogenesis is also recognized in the step of tumor progression, the final step of carcinogenesis that tumor cells acquire malignant phenotypes including metastatic properties. And the phenomenon is universally observed in several cell lines of different origins. In this review I would like to show the evidence that tumor develop ment and progression are accelerated inevitably by inflammation caused from foreign bodies, and that reactive oxygen species derived from inflammatory cells are one of the most important genotoxic mediators to accelerate the process.
© *2007 Wiley-Liss, Inc.*

Key words: inflammation; ROS; foreign body; carcinogenesis; tumor progression

The cancer research advanced in the last decade has pointed out that at least 5 cancer causing factors exist: spontaneous replication errors in DNA, cytotoxic and/or inflammatory carcinogenic substances, genotoxic (direct DNA injurious) carcinogenic substan ces, irradiation including ultra violet and transduction of viral oncogenes. Infection/inflammation is unquestionably among them. In 1981, cancer epidemiologists estimated that around 10% of can cers was due to infection/inflammation in the Unites States.[1] And the most recent statistics show the major involvement of inflammation in the total cancer death, at various proportions among countries; for example, in 2005, the ratio of infection/inflammation to all the cancer death causes was around 25% in sub Saharan Africa while that in Europe was around 6%.[2] In the year 2000, col lectively, 20% of all cancer deaths was attributable to inflammatory reactions due to chronic infections caused by infectious agents.[3]

In the same line of the evidence, foreign bodies incorporated into body for medical reasons or accidentally appear to lead to inflammation-based carcinogenesis. One of the epoch-making experimental studies in this aspect was established by Dr. Boone and his colleagues.[4 9] They observed tumorigenic conversion of immortalized cell lines after implantation of the cells attached to foreign bodies such as a piece of plastic plate or glass bead. Fol lowing their finding, we expanded the experiment to tumor progression, namely, the final stage of carcinogenesis acquiring malignant properties, and revealed that inflammatory-cell-derived reactive oxygen and nitrogen species were actually involved in the malignant progression. We also confirmed the universality of the phenomena in the cell lines of rat, mouse and human.

In the industrialized world, including developing and undevel oped countries alike, foreign body induced carcinogenesis tends to be poorly managed. A typical example is pleural mesothelioma among the particular workers in Japan; the disease is now belat edly being recognized after their years of exposure to asbestos in the past. Besides those, parasite related or environmental carcino genesis is among the foreign body induced carcinogenesis; how ever, in this article, I focus on the materials related with industrial products. I will present the past and present evidence for foreign body-carcinogenesis and demonstrate that host inflammatory reaction is one of the major factors promoting carcinogenesis.

Foreign body induced carcinogenesis in human

Exogenously incorporated foreign bodies can induce tumors in human. Various materials are implanted in human body for pros thetic, reconstructive or cosmetic purposes today; or accidental implantation occurs, such as bullets or shrapnel at war; further, nondigestible particles and scarring may be added to this category of foreign body.[10 12] Tumor latency period from such implanta tion/injury till its detection is extremely long. It is estimated that over 25% of tumors appears in the span of 15 years and 50%, 25 years,[11] and the overall latent period in human is said to be 20 years.[10] It should be noted, however, that despite the increasing use of medical implants over the last 6 decades the frequency of tumor development there is extremely low. The critical difference of foreign-body-induced carcinogenesis from inflammation-based carcinogenesis is the low or rare tumor incidence, although the incidence depends on the composition of foreign body.

Particulate carcinogens

Particulate carcinogens are carbon black, asbestos, diesel exhaust, coal particle, acid aerosols, tobacco smoke and reactive gases such as ozone, sulfur dioxide and nitrogen oxides, and the most steady mechanism of particulate carcinogens is to elicit intense inflammatory responses. The conspicuous feature of inflammation in particle-induced carcinogenesis is the release of inflammatory cytokines and reactive oxygen species (ROS).[13] On the other hand, other mechanisms are also reported[13,14]; for exam ple, such particles can act as carriers of carcinogen such as polycy clic aromatic hydrocarbons to increase retention period.[15]

Carbon black, a product of incomplete combustion of carbona ceous fuels and usually regarded as soot, was provisioned by Sir Percivall Pott in 1775 as a dreadful example of environmental car cinogen to cause scrotum tumors in chimney sweepers.[16] Soot itself appears to be carcinogenic on skin but possibly not on those of intracorporal organs such as respiratory tract epithelium.[17] A long term cohort study revealed that carbon black or related by products cause lung cancers in the workers at carbon black manu facturing factories.[18] Chronic exposure to particulate carcinogen increases tumor incidences to around 20%, with the majority of tumors appearing after 18 months of exposure.[19] While we are blessed with numerous products by technological innovations, regrettably some products are double edged, contrary to the origi nal aim of their production. The common features of the foreign bodies that induce carcinogenesis are listed in the later section. We need to pay attention to newly developed products before they

Grant sponsors: Japanese Ministry of Education, Culture, Sports, Sci ence and Technology; Japanese Ministry of Health, Labor and Welfare.
***Correspondence to:** Department of Biomolecular Function, Graduate School of Medical Science, Yamagata University, 2 2 2, Iidanishi, Yamagata 990 9585, Japan. Fax: 81 23 628 5230.
E mail: fuokada@med.id.yamagata u.ac.jp
Received 30 June 2007; Accepted after revision 9 August 2007
DOI 10.1002/ijc.23125
Published online 24 September 2007 in Wiley InterScience (www.interscience. wiley.com).

appear in the market. Nano scale particles, for example, are a great worriment because of the nature of their structures and those products are now spreading into every aspect of our daily life.[20]

Asbestos fibers

Asbestos is the commercial name for a group of hydrated mag nesium silicate fibrous minerals, which is used as materials of cement, ceiling, automobile brake linings, and shipbuilding for their characteristic resistance to heat and combustion.[21] Asbestos is in 3 types of crystals: chrysotile (white asbestos), crocidolite (blue asbestos) and amosite (brown asbestos). The carcinogenicity of asbestos is believed to be related with the structural type rather than physiochemical properties.[22] Imports of asbestos increased in Japan from early 1960 and had a peak in 1974,[23] although there was general warning in 1973 that inhalation of asbestos causes pneumoconiosis, bronchial carcinoma, carcinoma of the gastroin testinal tract and mesotheliomas.[13,24] In parallel with asbestos con sumption, the risk of developing mesothelioma (commonly pleu ral, and also peritoneal and pericardial mesotheliomas, depending on the distribution of mesothelium in body cavities) was belatedly recognized in people who had worked with asbestos. The mean la tency of mesothelioma development is estimated at 20 40 years after the first exposure to asbestos.[21,25]

When asbestos is experimentally injected into a subcutaneous space in mice, extensive inflammatory reaction develops; surpris-ingly the subcutaneously injected asbestos fibers are transported into submesothelium of thorax and abdomen.[26] The conspicuous feature of asbestos-associated mesotheliomas is persistent inflam-mation[27] and active fibrosis[28] evoked by asbestos fibers since immune system cannot remove the nondigestible particles that lead to chronic inflammation (also termed "sterile inflamma-tion).[29,30] It is considered that possible causes of asbestos induced mutation are ROS, reactive nitrogen species (RNS) and their by-products. Asbestos fibers themselves catalyze the forma-tion of hydroxyl radicals.[31,32] The production of hydroxyl radicals in cells after treated with crocidolite asbestos may result in the formation of premutagenic DNA basis, namely 8 hydroxy 2′ deoxyguanosine (8 OHdG).[33] Moreover, asbestos fibers stimulate infiltrated phagocytes to produce nitric oxide.[34] Thus in the environ ment of asbestos deposit site, RNS, in addition to generated ROS, may react with superoxide anion to produce peroxynitrites, which are able to oxidize guanosine to produce 8 OHdG. Unfried *et al*. have confirmed the evidence in *in vivo* situation that 8 OHdG dependent mutagenesis in the mesothelium (*i.e*., mostly G to T trans versions) occurred in rats after injection of crocidolite asbestos.[35]

In Japan, an exponential increase of deaths from pleural meso thelioma was observed in the early 1990 s. The mean annual num ber of deaths from malignant pleural mesothelioma was estimated at 500 between 1995 and 1999, and double fold in 2005.[21] Consid ering the long latency period of mesothelioma, the incidence is expected to increase further in the coming decades.[36]

Metals

Kawanishi *et al*. have proposed the hypothesis that carcinoge nicity of metal compounds is in 3 ways of inducing oxidative DNA damage.[37] First, metal generates ROS directly; second, ROS are generated through induction of inflammatory reaction by metal; third, metal activates carcinogenic chemicals. Inflamma-tion inducible metals are chromium (VI), cadmium, lead, cobalt (II), iron (III) and nickel compounds such as $NiSO_4$, NiO, Ni_3S_2 while Ni_3S_2 has a role to induce oxidative DNA directly in the presence of hydrogen peroxide.[37 39]

Medical devices

A wide variety of synthetic materials have been implanted in human since 1940 s. In the middle to late 1960 s, there were a few anecdotal reports, but they seldom mentioned the association between carcinogenicity and implantation of plastic materials. A long term investigation of cosmetic breast surgery (breast aug mentation) was the most typical example. Of 40,000 cases of im plantation of polyvinyl alcohol sponges, breast cancer developed only in 6 cases (0.015%).[40] A cohort study revealed that the over all mortality rate was lower in women with breast implants than women with other plastic surgery or general population.[41] And in breast cancer statistics, no differences were found between women with breast implants and those without them in the disease stage at diagnosis, cancer recurrence or survival time.[42,43] Moreover, breast implant after mastectomy did not affect cancer related mor tality.[44] It is acceptable and certainly safe to implant foreign body for medical purposes by today s technology. Nevertheless, there are chances that implantation of foreign body is not always safe; *e.g*., after arthroplasty of hip chromosomal aberrations are fre quently found in the adjacent bone marrow.[45]

Scar cancer and mechanical trauma/wound-healing-associated cancer

Scars reactive to or encapsulation of foreign bodies is the funda mental and common host reactions (also known as fibrosis, des-moplasia or stromal proliferation). In early 1940 s, G. Friedrich and R. Rössle found that lung carcinomas grew at the sites of fi-brous scars in patients with smoking habit.[46,47] They observed that the scars had preceded the cancer, and thus termed it scar cancer (Narbenkrebs). Encapsulation is the oldest host adaptive immune system, and all the living things, from invertebrates to vertebrates, are equipped with the system.[48] In man, it is commonly associated with pathological situations and the typically described is fibrous tissue capsule or scar which surrounds foreign bodies. Histological examination shows that fibrous capsule is generally 300-μm thick around the implanted materials; however, no association is observed between the implanted materials and the degree of cap sule reaction.[49] The initial pathognomonic sign is an emergence of extensively proliferating spots with a population of atypical cells, usually observed adjacent to a foreign body, and the cells are of polygonal and/or spindle cell types, expressing proliferating cell nuclear antigen.[49] These lesions can be considered as transitive to preneoplastic lesions.

There are 2 theories as for the induction of scar cancer. One is that the cells composing scar tissues convert themselves directly into tumorigenic ones; the other is that scar tissues secrete soluble factors that stimulate carcinogenic conversion or proliferation of tumor cells pre existing around the scar. The former theory is sup ported by the evidence that tumor nodules arise in the serial trans plantation of scar tissues which have originally been formed in the mice carrying T6 marker chromosomes; therefore by monitoring the chromosome as tag, one could detect the initial changes when the scar tissue exhibits conversion into malignant tumors at vari ous degrees in the scar environment.[50,51] At this point, the latter theory is more acknowledged, because scar formation occurs along with tumor development, rather than before tumor forma tion, as seen in lung adenocarcinoma development, for instance.[52] Scar can promote malignant growth of tumor cells; for this reason, the minimum take dose of scarred tissue is much smaller than that of unscarred tissue, for lethal growth of transplanted tumor cells.[53]

Mechanical trauma (post-traumatic inflammation) or wound-healing processes show a similar trend to scar cancer. In 1920 s a number of reports were published concerning the association between trauma/wound healing and cancer, and some of them were the reports of case studies, showing that the incidence of trauma could cause cancer.[54,55] Trauma is considered not to act as initiator, but to act as promoter of carcinogenesis.[56] Fibrosis asso ciated with tumor growth provides the environment to stimulate progression of tumor cells. Most of the clinical local recurrences of gastrointestinal tumors after resection of the primary tumor occur at the operated area of incision or anastomosis.[57,58] Whether fibrosis is accompanied with tumor or not depends on the types of collagen it produces.[59] Tumor-associated fibrosis is characterized by increased collagen Type III content; such fibrotic lesions are

mostly in the early immature stage. On the other hand, fibrosis unaccompanied with tumor contains decreased content of Type III and increased contents of Types I and IV collagen; such fibrotic lesions are mostly in the late mature stage.[60]

Foreign body induced experimental carcinogenesis

In 1941, Turner described the first experimental evidence for foreign body induced sarcoma in rat using a disk of Bakelite[61] which was the first plastic made from synthetic components.[62] Brand *et al*. verified that carcinogenic potential depended on the properties of foreign body such as shape/size, smoothness, hardness, porosity and electrostatic load,[63] and was also influenced by gender and strain of the host.[64] Oppenheimer revealed that the tumor forming material was not of degradable nature and had enduring smooth surface,[65] which was thereafter referred to as Oppenheimer effect. [66,67] The effect is also called solid state tumorigenesis because the carcinogenic conversion is brought about on solid surface of a foreign body.

The shape necessary for carcinogenesis as found in foreign bodies must be *in vivo* as well. An easily absorbable liquid would provide little or no chance for the conversion; however, if the liquid turns viscous, it can produce fibrosarcoma at the site of implantation.[67] The shape also influences tumor frequency; it is much higher in mice implanted with concave plastic discs than in those with convex ones because concave discs evoke more intense fibroblastic reaction.[10] Moreover, tumor incidence correlates with surface area of implants because the size of foreign bodies determines the degree of the inflammatory reaction.[10] Tumor formation seldom occurs when a small material has been implanted, but a large foreign body produces tumors constantly.[68] Textile materials, rough surfaced implants and minced materials have little potential to induce tumors, whereas, if they are implanted, uninterrupted and smoothly surfaced, they develop tumors.[10,28,69]

Porosity of the materials is influential. When implants are perforated and the holes are large, tumor incidence is reduced.[10,68] For instance, filters with pores of 0.22 μm or larger do not induce tumors whereas those of the same thickness with smaller pores will do. This is because the implants poorly develop connective tissue capsules and the pore is infiltrated with phagocytes in the former,[10] but the pore sizes below 0.22 μm are surrounded by thick collagen capsules and the filter pores are not infiltrated with phagocytes. The latter fibrous capsules are highly vascularized and the capillary loops develop close to the implant surface (less than 30 μm); on the other hand, angiogenic response is suppressed in the area of nontumorigenic filter implantation and the mean distances between the implant surface and capillaries remain 300 μm.[10] The filter with large porosity was found to have less cell proliferation, apoptosis, and fibrosis.[70] Moreover, hydrophobic filters develop more tumors than mice implanted with hydrophilic ones.[10] The electrical effects, interfacial forces or electrostatic and/or electrokinetic imbalance of implanted materials affect carcinogenic incidence.[53,69] Foreign materials positive for electric charge spontaneously attract inflammatory cells or easily form thrombus because most inflammatory cells, especially neutrophils, are negative for electric charge.[28]

It is of interest to know what cell types are responsible for foreign body induced carcinogenesis. There are 3 candidates, macrophages, fibroblasts and pericytes.[10] However, it would be appropriate to exclude macrophage as the early experiment suggested its irrelevance. Namely, in the experiment of bone marrow macrophage transplantation using distinguishable syngeneic mice, genetically marked sarcoma was not found in the mice whose bone marrow had been replaced to genetically tagged marrow cells.[10] Histologically the predominant cell type to form capsules around implants is fibroblast while foreign body-induced tumors are predominantly sarcomas. Fibroblast is a dubious entity. While development of fibrosarcoma is dominant in chemically-induced carcinogenesis, a variety of sarcomas appear in foreign body induced carcinogenesis: fibrosarcomas, malignant fibrous histiocytomas, pleomorphic sarcomas, myxosarcomas, hemangiosarcomas, rhabdomyosarcomas, osteosarcomas, leiomyosarcomas and mixed type.[49] This may illustrate that the initial mesenchymal stem cell reactive to foreign body is pluripotent. Pericyte is another candidate since its initial role is to support local angiogenesis. However, because of their pluripotent potential as a local mesenchymal cell population, they can convert themselves into sarcomas with various histological characteristics.[10]

Genetic alterations in the tumors arisen in the foreign body implantation have extensively been analyzed initially by karyotyping. Derivations from the normal chromosome number are seen in all the tumors studied[10]; morphological aberrations of chromosomes such as metacentrics or double minutes, and conspicuous abnormalities are recognized. Most karyotypes of foreign body induced tumors are exhibited in the hyperdiploid or the hypo to hyper tetraploid ranges.[10] Tazawa *et al*. identified some genes possibly responsible for the development of foreign body induced sarcomas.[71] They found that in 79% heterozygous p53-deficient ($Trp53^{+/-}$) mice with only 1 functional allele in the p53 gene developed spontaneous sarcomas after plastic plate implantation, whereas only 10% of mice with wild type p53 developed sarcomas. They further demonstrated that the arising tumors have lost the remaining wild type p53 allele, meaning complete loss of p53 function; this appears to be the underlying molecular mechanism during the development of sarcomas. That is, the tumor suppressor gene p53 is one of the genes responsible for foreign body induced carcinogenesis. p53 allele is inactivated by an increase of inflammation mediated RNS.[71] Association between RNS and p53 mutations is evidenced in inflamed lesions of the colon with ulcerative colitis,[72] stomach,[73] brain[74] and breast.[75]

The susceptibility to foreign body related carcinogenesis does exist across species, and in strain and gender dependent manner.[10] Carcinogenicity of foreign bodies in human is rare, especially that of clinically used materials such as polyethylene, polyurethane, polyvinyl chloride, polymethylmethacrylate, silicone, titanium, nickel chromium, cobalt chromium alloy and aluminum oxide; however, the same materials are indeed responsible for 25.8% incidence of sarcomas in 490 rats examined.[49] There is no association between histological types of sarcoma and implanted foreign materials, although polyurethane is the only material which tends to form hemangiosarcomas.[49]

Experimental rodent animals, except guinea pigs, exhibit high incidences of foreign body induced carcinogenesis.[11] Yet differences do exist among the strains of mice in the incidence and the latency period.[64] And individuals genetically sensitive to foreign body induced tumors do exist in certain areas,[76] indicating differences in the sensitivity among races or groups of inhabitants. It is expected that there are labile gene(s) for this. Gender differences are also exhibited in mice. Latency period of tumor development is shorter in female mice than in male mice; however, the malignancy of the arising tumors is much enhanced in male mice.[10] By comparing the differences in sensitivity or resistance to foreign body related carcinogenesis we might be able to find a clue to identify the molecules that control foreign body related carcinogenesis.

Experimental models of foreign body induced carcinogenesis and tumor progression

Serendipitous discovery of the foreign body induced carcinogenesis was made by Dr. Boone s group.[4 6] They showed that cells of nontumorigenic but immortalized mouse cell lines or freshly isolated connective tissues from mice were converted into lethally growing tumors of monoclonal origin in mice after they were implanted, being attached to foreign bodies.[4,5,8,9] They suggested at least 5 causes for the conversion: (*i*) immortalized cells acquired preneoplastic phenotype for the lack of anchorage independent growth property, and thus the substrate attached cells grew exponentially *in vitro* and lethally *in vivo*[4,5]; (*ii*) activation of endogenous oncornaviruses. However, this possibility was

low[6]; (*iii*) culture medium contained carcinogenic substances[7]; (*iv*) culture dish secreted carcinogenic substances.[8] Heppner, one of their contemporary researchers, speculated that inflammation was one of the possible causative factors for foreign body induced tu morigenesis.[77] Since then, we have expanded the experimental sys tems and demonstrated that foreign-body-induced inflammation and its-derived ROS are definitely the cause for the conversion.

To establish the experimental model of conversion, we chose the cells which were weakly or nontumorigenic and nonmeta static, but would grow *in vitro*, bearing the idea of xenogenization in mind. Xenogenization of tumor cells is the term meaning immunological spontaneous regression of tumor cells which had been infected with xenogeneic viruses,[78] transfected with the genes coding allogeneic antigen,[79] or exposed to mutagenic chem icals,[80] after injected into normal syngeneic host. Another approach to establish the model is to obtain the culture cell lines from a precancerous lesion.

Before I detail our unique animal models in which we can con sistently observe not only tumor development but also malignant progression of regressive tumors or precancerous cells originated from rat, mouse and human, I should briefly trace the history of our experiments. Following Dr. Boone s work, Drs. Takeichi and Hamada expanded the concept of foreign body induced carcino genesis to foreign body induced tumor progression. They obtained weakly tumorigenic and nonmetastatic clonal ER 1 cells by exposing SST 2 culture cell line, which had been established from a spontaneous mammary adenocarcinoma developed in SHR rat, to ethyl methanesulfonate *in vitro*.[81] The ER 1 cells regressed spontaneously after injected into syngeneic normal rats; however, if they were implanted, being attached to plastic plates (polysty rene, used as culture dish), into a subcutaneous space in rats, they acquired not only tumorigenicity but also metastatic ability.[81] The tumors arisen in rats showed various malignant phenotypes and their acquired phenotypes were stable. Thus they found that chronic inflammation was required for the progression of ER-1 cells. This model mimics inflammatory breast cancer in human, the most aggressive form of primary breast carcinoma with a dis mal outcome despite multimodal treatment approaches.[82]

They also determined that fibrous stroma secreted soluble fac-tors such as epidermal growth factor (EGF) and transforming growth factor (TGF).[83] The ER 1 cells were turned into inva sive and metastatic tumor cells by EGF continuously added into the culture.[84] Those phenotypic changes depended on the duration of the EGF treatment; the malignant features were reversible dur ing the 24 hr exposure to EGF, and after more than 4 weeks of ex posure the acquired malignant phenotypes were completely fixed.[84] It has been confirmed that acquisition of malignant pheno-types can be prevented by addition of antioxidants, *N* acetylcys tein or selenium, into the medium,[84,85] and that 8 OHdG, a marker of ROS mediated nucleic acid damage, is formed in the EGF treated ER 1 cells; therefore, it is strongly suggested that the fun damental cause for the malignant progression is ROS generated by the fibrous stroma-derived growth factors.[84]

The mouse model has thoroughly been investigated, and reveals a definite connection between foreign-body-induced inflammation and carcinogenesis. For further experiments, we obtained regres sive clonal QR cells by exposing clonal fibrosarcoma cells, BMT-11 cl 9, to a mutagen, quercetin *in vitro*.[80] Since QR cells grew progressively in immunosuppressed hosts, their regression was mediated by host immunity.[86] QR cells did not form tumors or metastasis after subcutaneous (2×10^5 cells) or intravenous (1×10^6 cells) injection into mice.[80] However, implantation of 1×10^5 QR cells attached to plastic plate[87] or injection into the preinserted gelatin sponge[88] in subcutaneous space of mice induced lethal tumors. Moreover, the arising tumor exhibited metastatic proper ties. The acquired various malignant phenotypes remained stable as far as examined for 1 year at least under cultivation *in vitro*.

We detected several gene alterations through the malignant con version in this model.[89] The level of thymosin 4 gene, which is known as an actin regulating protein and to function for angiogen esis and wound healing,[90] was elevated in all of the arising tumor cells. From the results of sense and antisense cDNA transfection experiments, we determined that thymosin 4 gene was responsi ble for tumor metastasis through regulating cell motility.[91] The expression of E1AF, a member of the *ets* oncogenic transcription factor, was found high in the arising tumor lines.[92] E1AF regulates tumor cell motility and invasive activities through induction of membrane type 1 matrix metalloproteinase which converts the latent form of matrix metalloproteinase 2 into active form.[92] Thus E1AF makes tumor cells invasive.[92]

We used two foreign bodies in our decades of experiments. One was a piece of plastic plate and the other a piece of gelatin sponge which is used as hemostasis material during surgical operation. The difference between the foreign bodies is in the capacity to elicit inflammation. Plastic plate initially induces acute inflamma-tion, which then changes to chronic inflammation, whereas gelatin sponge induces mild inflammation, and about 4 weeks after im-plantation it is naturally absorbed[93]; therefore such transition from acute to chronic inflammation is unlikely to occur in the use of sponge.[88] By using those foreign bodies, we modulated the quality and duration of the inflammation, and found that the type of inflammation needed for QR cells' growth and progression was acute-phase inflammatory reaction.[87,88,94] By histological exami nation, we found that neutrophils predominantly infiltrated the sponge.[93,94] In fact, one of the benefits of using gelatin sponge is that it is possible to collect the infiltrated inflammatory cells by treating the sponge with collagenase; the inflamed cells separated from the sponge can convert QR cells into tumorigenic ones if both cells are mixed and injected.[88,94] Namely, our primary obser vation was that foreign-body-reactive, early-phase inflammatory cells contribute to augment malignancy of QR cells.[87,88,94]

To test the role of neutrophils during inflammation, we elimi-nated neutrophils by administering anti neutrophils antibody (RB6). Nearly all the arising tumors in the mice, nontreated or treated with control rat IgG, acquired malignant phenotypes. On the other hand, RB6 antibody administered mice did not acquire malignant phenotypes.[94] We confirmed the results in integrin-β 2 knockout mice (C57BL/6J^{Itgb2tm1Bay} equivalent to CD18 deficient).[94] Integrin β 2 is the key adhesion molecule for the migration of neutrophils into an inflammatory region. Neutrophil infiltration into gelatin sponge is abolished and acquisition of ma-lignant phenotypes is suppressed in the integrin β 2 knockout mice.[94] These findings show that neutrophils are one of the main components of inflammation-associated tumor development and progression. Interestingly, the capability of neutrophils to acceler ate tumor cell malignancy depends on their activation phase since circulating or bone marrow neutrophils do not convert regressive tumor cells into malignant ones.[94]

It is reported that tumor-infiltrated neutrophils have the role to induce angiogenesis *via* MMP 9 secretion.[95] We assumed that the neutrophils also produced genotoxic substances since we detected somatic gene mutation in the coculture of QR cells and the infil-trated neutrophils.[96] The somatic mutation was inhibited in the presence of a radical scavenger, mannitol[96]; and in our previous study, immunostaining of 8 OHdG in the tumor tissues evidenced neutrophils' infiltration.[93,94] Therefore we examined the roles of neutrophil derived ROS in this model. To determine the direct contribution of ROS to carcinogenesis, we used gp91phox gene knockout mice. Bactericidal function of neutrophils brought about generation of superoxide anions by forming NADPH oxidase complex (gp22^{phox}, gp40^{phox}, gp47^{phox}, gp67^{phox}, gp91^{phox} and Rac1/Rac2) from interaction with cytochrome b_{558}.[97] The fre quency of tumor development from the QR cells coimplanted with gelatin sponge was decreased in the gp91$^{phox-/-}$ mice. Moreover, acquisition of metastatic ability was reduced in the mice.[98] Thy mosin 4, a genetic marker of QR cell progression,[91] was not detected in the arising tumors in gp91$^{phox-/-}$ mice. To confirm whether phagocyte derived ROS were actually involved in tumor progression, we isolated phagocytes from wild type mice and

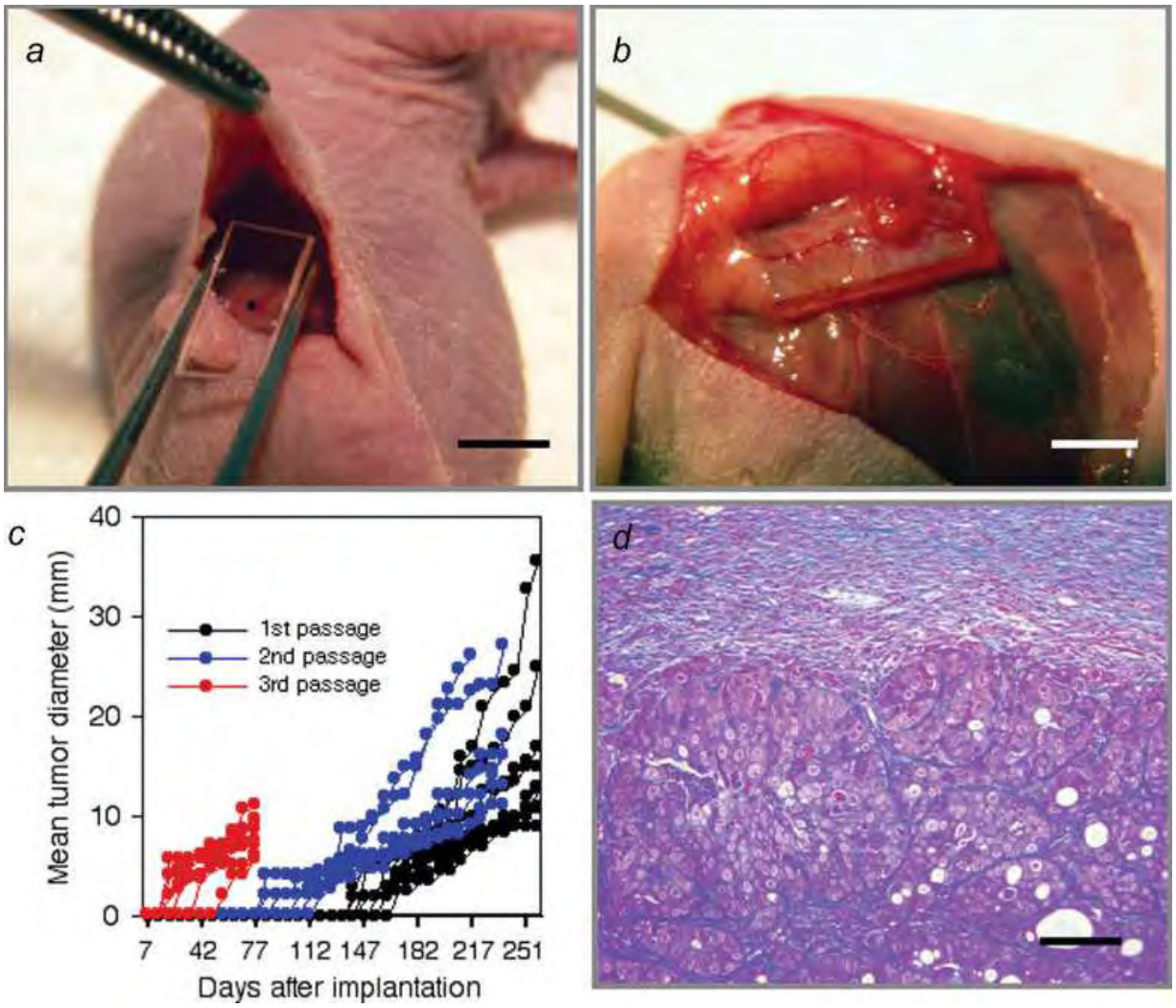

FIGURE 1 Features of the arising adenocarcinoma after implantation of human colonic adenoma cells being attached to plastic plate. (*a*) Ad enoma cells (1×10^5 cells per plate) were implanted into a subcutaneous space of nude mouse. Bar, 5 mm. (*b*) Around 5 months later, a palpable mass with forming angiogenesis appeared; the emergence was always monoclonal growth. Bar, 5 mm. (*c*) Latency period was shortened after se rial transplantation of the arising adenocarcinoma cells with plastic plate attached. (*d*) Histologically the arising tumors were moderately differ entiated adenocarcinoma with proliferating stroma (Azan staining). Bar, 100 μm.

transferred them into $gp91^{phox-/-}$ mice. As a result, wild type derived phagocytes increased the frequencies of tumor develop ment and metastasis. In contrast, the phagocytes obtained from knockout mice did not have such activity.[98] Moreover, administra tion of aminoguanidine, a broad inhibitor for inducible nitric oxide synthase, partially but significantly suppressed malignant conver- sion in the model[93]; thus we concluded that RNS were also involved in the process. These results show that ROS and RNS, derived from foreign body induced neutrophils, are an intrinsic factor in the conversion of regressive tumors to more malignant ones. The mouse model may recapitulate the typical inflamma- tion based carcinogenesis, and thus be suitable for analyzing bio logical causes of the process. Gelatin sponge implantation induces massive and persistent infiltration of activated leucocytes; such situation may provide pathogenic resemblance to the continuous infiltration of activated inflammatory cells into target organs by bacteria (*Helicobacter pylori*[99]) or parasites (*Opistorchis* sp., *Chlonorchis* and *Schistosoma*[100]) infections in human.

Since we used rodent cells previously, we have been eager to prove the phenomena by using cells of human and epithelial ori gin. For this purpose, instead of establishing regressive tumors from lethally growing tumors, we have picked up an available cell line derived from precancerous tissues of colon. In patients with ulcerative colitis or Crohn's disease, a typical inflammation- related carcinogenesis is seen; that is, continuous infiltration of disordered immune cells into the autologous colon tissue precedes the development of colorectal cancer.[101,102] A culture cell line of adenoma cells (FPCK 1 cells) was established by Dr. Kawaguchi from a colonic polyp of a patient with familial adenomatous poly posis.[103] The phenotype of the cell line was stable and no sponta neous conversion was observed during the maintenance under reg ular cultivation at least for 1 and half years.

We used several sister lines of adenoma FPCK 1; they did not grow in nude mice when injected at 5×10^6 cells in a suspension form. However, adenoma cells of FPCK 1 1 formed palpable tumors in about 5 months when they were attached to plastic plate and implanted into subcutaneous space of mice (Fig. 1*a*).[104] Their tumorigenic growth always started from 1 colony (monoclonal ori gin) (Fig. 1*b*). Serial implantation of the culture cell line with plas tic plate, established originally from the arising tumor after

TABLE I UNIVERSALITY OF CARCINOGENESIS AND ITS PROGRESSION INDUCED BY FOREIGN BODIES IN THE CELLS DERIVED FROM IMMORTALIZED CELLS, REGRESSIVE TUMOR CELLS OR PRECANCEROUS LESIONS

Cells	Origin of the cell	Implantation host	Foreign body	Characteristics of arising tumors (reference)	Year
BALB/3T3	mouse, vascular endothelium	mouse	glass beads	malignant hemangioendothelioma (4)	1975
NCTC 8467	mouse lung tissue	mouse	glass helices	sarcoma (105)	1980
BALB/3T3	mouse, vascular endothelium	mouse	polycarbonate plastic plate	vasoformative sarcoma (5)	1976
C3H/10T1/2	mouse, embryo	mouse	polycarbonate plastic plate	fibrosarcoma (5)	1976
C3H/10T1/2	mouse, embryo	mouse	polycarbonate plastic plate	invasive fibrosarcoma (9)	1979
Connective tissues obtained from adult mice		mouse	polycarbonate plastic plate	undifferentiated sarcoma (8)	1979
ER 1	rat, regressive mammary adenocarcinoma	rat	polystyrene plastic plate	malignant, sarcomatoid histology (81)	1992
QR 32	mouse, regressive fibrosarcoma	mouse	polystyrene plastic plate	malignant fibrosarcoma (87)	1993
FPCK 1 1	human, colonic adenoma	mouse	polystyrene plastic plate	high tumor incidence, adenocarcinoma (104)	2000
Benzo(a)pyrene induced sarcoma		mouse	polystyrene plastic plate	shortened latency period (112)	2002
Subcutaneous space of mice		p53 heterozygous mouse	polystyrene plastic plate	high tumor incidence, fibrosarcoma (72)	2007
Subcutaneous space of mice		p53 wild type mouse	polystyrene plastic plate	low tumor incidence, fibrosarcoma (72)	2007
QR 32	mouse, regressive fibrosarcoma	mouse	gelatin sponge	malignant fibrosarcoma (88)	1992
Lk9dL	rat, nonmetastatic renal carcinoma	rat	gelatin sponge	metastatic renal carcinoma (107)	1998
FPCK 1 1	human, colonic adenoma	mouse	gelatin sponge	low tumor incidence, adenocarcinoma (104)	2000

implantation of the adenoma cells with plastic plate, shortened the latency period, in correlation with the number of passage (Fig. 1*c*).

Histologic examination revealed that the arising tumors were moderately differentiated adenocarcinoma, and they were sur rounded by highly collagenic fibrous stroma (Fig. 1*d*). The fibrous tissue, rather than attachment to the plastic plate substrate, was considered essential for the conversion, because we observed tu mor growth after injection of the adenoma cells directly at the site of proliferating stromal tissues where the plastic plate had been implanted for about 5 months and then removed. In contrast, there was no tumor development in nontreated or sham operated mice, and tumor seldom arose after coimplantation with gelatin sponge in those, which indicated that acute inflammation did not suffice to convert the adenoma cells.[104] The stromas at the chronic inflammatory region secrete specific soluble factor(s), which have not been identified yet; we assume that the factor(s) could stimulate adenoma cell growth but not the growth of adenocarcinoma cells. Such factors could not be produced either from normal subcutane ous fibroblasts or immortalized mouse fibroblast cell lines.[104]

In our foreign body induced carcinogenesis and tumor progres sion models, we can observe that inflammation promotes malignancy of various species of cells. As summarized in Table I, the phenomenon is universally observed among species and in tissues of different origins.

Perspective of prevention of foreign body induced carcinogenesis

Inflammatory environments due to the existence of foreign body cause a variety of biological responses as they contain increased growth/survival factors, chemotactic cytokines (chemo kines), matrix metalloproteases, adhesion molecules, extracellular matrix, inflammatory mediators (*i.e.*, histamine, eicosanoids, inflammatory cytokines and proteases), DNA-damage-promoting agents (*i.e.*, ROS and RNS) and augmented angiogenesis[108] (Fig. 2). I have been emphasizing the roles of inflammatory-cell-derived ROS in the foreign body induced carcinogenesis. In this section, I would like to suggest some strategies to prevent it. If the inflammatory cell derived ROS are critical to promote foreign body induced carcinogenesis, the sensitivity of cells to the conversion should be controlled by intracellular or tissue antioxidative poten tials. Our earlier study demonstrated that a variation was observed among the QR clones in the frequency of tumor progression by coimplantation with foreign body.[96] We determined that the varia tion was due to intracellular antioxidative enzymes, manganese superoxide dismutase, Mn SOD and glutathione peroxidase, GPx level in the QR clone. An inverse correlation was observed between the contents/activities of those enzymes and the sensitiv ity of QR clones to progress under foreign-body-induced inflammatory environment.[96] Thus, we determined that cells with low antioxidative levels are prone to convert themselves into more ma lignant ones in the presence of inflammation and the inflammatory cell derived ROS. In other words, foreign body induced carcino genesis in QR cells will be prevented if the cells have an adequate amount of antioxidative enzymes or induction of the enzymes at the implantation site. The prevention was actually achieved by induction of Mn SOD at the coimplantation site by administering Mn-SOD-inducible biological response modifier[109] or orally avail able superoxide dismutase in this system.[110] Moreover, it is assumed that RNS are partially involved in the model, because administration of a broad inhibitor for inducible nitric oxide sig nificantly inhibited inflammation-induced tumor progression.[93]

By extensively analyzing human tissue materials obtained from typical inflammation-based carcinogenesis, it has been revealed that ROS and RNS are inevitably involved in the development and progression of tumors.[111,112] Considering those clinical studies

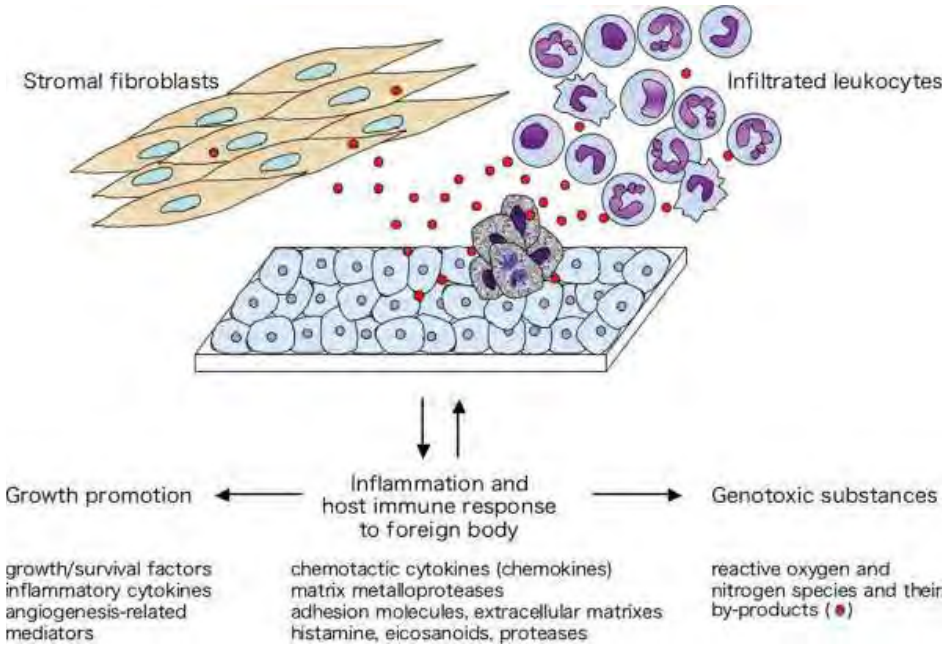

FIGURE 2 Key biologic features of foreign body induced carcinogenesis. Malignant conversion of precancerous cells or progression of regressive tumor cells requires foreign body induced inflammatory soluble mediators. The mediators can be classified as (*i*) growth promotion, (*ii*) inflammation and host immune response to foreign body and (*iii*) genotoxic substances (*i.e.*, reactive oxygen and nitrogen species). Each precancerous cell or regressive tumor cell possibly carries a variety of gene alterations. And only one of the cells may undergo the final crucial molecular changes that convert/progress to malignant one. Such clone originated cell acquires various malignant phenotypes during proliferation under the influence of inflammatory soluble mediators.

and our results, we conclude that the proneness of tumor cells to be more malignant depends on the balance between antioxidative enzyme activities in themselves and the duration/amount of ROS and RNS generated by inflammatory cells, and that disturbing the balance will be the most effective strategy for the prevention of foreign body induced carcinogenesis.

References

1. Doll R, Peto R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. J Natl Cancer Inst 1981;66:1191–308.
2. World Health Organization and International Union Against Cancer. Global action against cancer updated version. Geneva: WHO, 2005.12.
3. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol 2001;2:533–43.
4. Boone CW. Malignant hemangioendotheliomas produced by subcutaneous inoculation of Balb/3T3 cells attached to glass beads. Science 1975;188:68–70.
5. Boone CW, Jacobs JB. Sarcomas routinely produced from putatively nontumorigenic Balb/3T3 and C3H/10T1/2 cells by subcutaneous inoculation attached to plastic platelets. J Supramol Struct 1976;5:131–7.
6. Boone CW, Takeichi N, Paranjpe M, Gilden R. Vasoformative sarcomas arising from BALB/3T3 cells attached to solid substrates. Cancer Res 1976;36:1626–33.
7. Takeichi N. Spontaneous transformation of in vitro normal cells. Hokkaido Igaku Zasshi 1978;52:353–4.
8. Boone CW, Takeichi N, Eaton SD, Paranjpe M. Sontaneous neoplastic transformation in vitro: a form of foreign body (smooth surface) tumorigenesis. Science 1979;204:177–9.
9. Boone CW, Vembu D, White BJ, Takeichi N, Paranjpe M. Karyotypic, antigenic, and kidney invasive properties of cell lines from fibrosarcomas arising in C3H/10T1/2 cells implanted subcutaneously attached to plastic plates. Cancer Res 1979;39:2172–8.
10. Brand KG. In: Symington T, Carter RL, eds. Scientific foundations of oncology. London: Wm Heineman Med Book Ltd, 1976.490–95.
11. Brand KG, Brand I. Risk assessment of carcinogenesis at implantation sites. Plast Reconstr Surg 1980;66:591–5.
12. Brand KG. In: Becker FF, ed. Cancer: a comprehensive treatise, 2nd edn., vol. 1. New York: Plenum Press, 1982.661–92.
13. Borm PJ, Driscoll K. Particles, inflammation and respiratory tract carcinogenesis. Toxicol Lett 1996;88:109–13.
14. Belinsky SA, Snow SS, Nikula KJ, Finch GL, Tellez CS, Palmisano WA. Aberrant CpG island methylation of the p16(INK4a) and estrogen receptor genes in rat lung tumors induced by particulate carcinogens. Carcinogenesis 2002;23:335–9.
15. Nettesheim P, Topping DC, Jamasbi R. Host and environmental factors enhancing carcinogenesis in the respiratory tract. Annu Rev Pharmacol Toxicol 1981;21:133–63.
16. Pott P. Chirurgical observations relative to the cataract, the polypus of the nose, cancer of the scrotum, different kinds of ruptures, and the modification of the toes and feet. London Hawes 1775;1–208.
17. Falk HL, Steiner PE. The identification of aromatic polycyclic hydrocarbons in carbon blacks. Cancer Res 1952;12:30–9.
18. Sorahan T, Harrington JM. A lugged analysis of lung cancer risks in UK carbon black production workers, 1951–2004. Am J Ind Med 2007;50:555–64.
19. Nickell Brady C, Hahn FF, Finch GL, Belinsky SA. Analysis of Kras, p53 and c raf 1 mutations in beryllium induced rat lung tumors. Carcinogenesis 1994;15:257–62.
20. Chen H W, Su S F, Chien C T, Lin W H, Yu S L, Chou C C, Chen JJW. Titanium dioxide nanoparticles induce emphysema like lung injury in mice. FASEB J 2006;20:2393–5.
21. Maeda M, Hino O. Molecular tumor markers for asbestos related mesothelioma: serum diagnostic markers. Pathol Int 2006;56:649–54.
22. Stanton MF. Fiber carcinogenesis: is asbestos the only hazard? J Natl Cancer Inst 1974;52:633–4.
23. Morinaga K, Kishimoto T, Sakatani M, Akira M, Yokoyama K, Sera Y. Asbestos related lung cancer and mesothelioma in Japan. Ind Health 2001;39:65–74.
24. Nizze H. Exposure to asbestos and the genesis of pleural plaques and neoplasia. Arch Pathol 1973;95:213–4.
25. Selokoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. JAMA 1964;188:22–6.
26. Roe FJ, Carter RL, Walters MA, Harington JS. The pathological effects of subcutaneous injections of asbestos fibres in mice: migration of fibres to submesothelial tissues and induction of mesotheliomata. Int J Cancer 1967;2:628–38.
27. Wagner JC. Experimental production of mesothelial tumours of the pleura by implantation of dusts in laboratory animals. Nature 1962;196:180–1.
28. Rigdon RH. Tissue reaction to foreign materials. CRC Crit Rev Toxicol 1975;3:435–76.
29. Steenland K, Stayner L. Silica, asbestos, man-made mineral fibers, and cancer. Cancer Causes Control 1997;8:491–503.
30. Shacter E, Weitzman SA. Chronic inflammation and cancer. Oncology 2002;16:217–32.
31. Weitzman SA, Graceffa P. Asbestos catalyzes hydroxyl and superoxide radical generation from hydrogen peroxide. Arch Biochem Biophys 1984;228:373–6.

32. Maples KR, Johnson NF. Fiber induced hydroxyl radical formation: correlation with mesothelioma induction in rats and humans. Carcino genesis 1992;13:2035 9.
33. Fung H, Kow YW, van Houten B, Mossman BT. Patterns of 8 hydroxydeoxyguanosine formation in DNA and indications of oxida tive stress in rat and human pleural mesothelial cells after exposure to crocidolite asbestos. Carcinogenesis 1997;18:825 32.
34. Thomas G, Ando T, Verma K, Kagan E. Asbestos fibers and inter- feron gamma up regulate nitric oxide production in rat alveolar mac rophages. Am J Respir Cell Mol Biol 1996;11:707 15.
35. Klaus U, Claudia S, Josef A. Distinct spectrum of mutations induced by crocidolite asbestos: clue for 8 hydroxydeoxyguanosine dependent mutagenesis *in vivo*. Cancer Res 2002;62:99 104.
36. Robinson BWS, Richard A, Lake RA. Advances in malignant meso thelioma. N Engl J Med 2005;353:1591 603.
37. Kawanishi S, Hiraku Y, Murata M, Oikawa S. The role of metals in site-specific DNA damage with reference to carcinogenesis. Free Radic Biol Med 2002;32:822 32.
38. Kasprzak KS. Possible role of oxidative damage in metal induced car cinogenesis. Cancer Invest 1995;13:411 30.
39. Kawanishi S, Inoue S, Oikawa S, Yamashita N, Toyokuni S, Kawa nishi M, Nishino K. Oxidative DNA damage in cultured cells and rat lungs by carcinogenic nickel compounds. Free Radic Biol Med 2001;31:108 16.
40. Hoopes JE, Edgerton MT, Jr, Shelley W. Organic synthetics for aug mentation mammaplasty: their relation to breast cancer. Plast Reconstr Surg 1967;39:263 70.
41. Villeneuve PJ, Holowaty EJ, Brisson J, Xie L, Ugnat AM, Latulippe L, Mao Y. Mortality among Canadian women with cosmetic breast implants. Am J Epidemiol 2006;164:334 41.
42. Clark CP, Peters GN, O Brien KM. Cancer in the augmented breast. Diagnosis and prognosis. Cancer 1993;72:2170 4.
43. Skinner KA, Silberman H, Dougherty W, Gamagami P, Waisman J, Sposto R, Silverstein MJ. Breast cancer after augmentation mammo plasty. Ann Surg Oncol 2001;8:138 44.
44. Le GM, O Malley CD, Glaser SL, Lynch CF, Stanford JL, Keegan TH, West DW. Breast implants following mastectomy in women with early stage breast cancer: prevalence and impact on survival. Breast Cancer Res 2005;7:R184 93.
45. Case CP, Langkamer VG, Howell RT, Webb J, Standen G, Palmer M, Kemp A, Learmonth ID. Preliminary observations on possible prema lignant changes in bone marrow adjacent to worn total hip arthro plasty implants. Clin Orthop Relat Res 1996;329:S269 79.
46. Friedrich G. Periphere Lungenkrebse auf dem Boden pleuranaher Narben. Virchows Arch Pathol Anat 1939;304:230 47.
47. Rössle R. Die Narbenkrebse der Lungen. Schweizerische Medizini sche Wochenschrift 1943;39:1200 3.
48. Qin Z, Kim HJ, Hemme J, Blankenstein T. Inhibition of methylcho lanthrene induced carcinogenesis by an interferon gamma receptor dependent foreign body reaction. J Exp Med 2002;195:1479 90.
49. Kirkpatrick CJ, Alves A, Köhler H, Kriegsmann J, Bittinger F, Otto M, Williams DF, Eloy R. Biomaterial induced sarcoma: a novel model to study preneoplastic change. Am J Pathol 2000;156:1455 67.
50. Brand KG, Buoen LC, Brand I. Foreign body tumorigenesis induced by glass, and smooth, and rough plastic: comparative study of preneo plastic events. J Natl Cancer Inst 1975;55:319 22.
51. Brand KG, Buoen LC, Brand I. Multiphasic incidence of foreign body induced sarcomas. Cancer Res 1976;36:3681 3.
52. Shimosato Y, Suzuki A, Hashimoto T, Nishiwaki Y, Kodama T, Yoneyama T, Kameya T. Prognostic implications of fibrotic focus (scar) in small peripheral lung cancers. Am J Surg Pathol 1980;4:365 73.
53. Moizhess TG, Vasiliev JM. Early and late stages of foreign body car cinogenesis can be induced by implants of different shapes. Int J Can cer 1989;44:449 53.
54. Dolberg DS, Hollingsworth R, Hertle M, Bissell MJ. Wounding and its role in RSV mediated tumor formation. Science 1985;230:676 8.
55. Weiss L. Some effects of mechanical trauma on the development of primary cancers and their metastases. J Forensic Sci 1990;35:614 27.
56. Kotin P, Kahler JE. Possible role of trauma as a cocarcinogen; case report on an oil worker. Cancer 1953;6:266 8.
57. Cass AW, Million RR, Pfaff WW. Patterns of recurrence following surgery alone for adenocarcinoma of the colon and rectum. Cancer 1976;37:2861 5.
58. Domellof L, Janunger KG. The risk for gastric carcinoma after partial gastrectomy. Am J Surg 1977;134:581 4.
59. van den Hooff A. Stromal involvement in malignant growth. Adv Cancer Res 1988;50:159 96.
60. Madri JA, Carter D. Scar cancers of the lung: origin and significance. Hum Pathol 1984;15:625 31.
61. Turner FC. Sarcomas at sites of subcutaneously implanted Bakelite disks in rats. J Natl Cancer Inst 1941;2:81 3.
62. http://acswebcontent.acs.org/landmarks/newproducts_t.html#bakelite
63. Brand KG, Buoen LC, Brand I. Foreign body tumorigenesis by vinyl chloride vinyl acetate copolymer: no evidence for chemical carcino genesis. J Natl Cancer Inst 1975;54:1259 62.
64. Brand I, Buoen LC, Brand KG. Foreign body tumors of mice: strain and sex differences in latency and incidence. J Natl Cancer Inst 1977;58:1443 7.
65. Oppenheimer BS, Oppenheimer ET, Danishefsky I, Stout AP. Carci nogenic effect of metals in rodents. Cancer Res 1956;16:439 41.
66. Bischoff F, Bryson G. Carcinogenesis through solid state surfaces. Prog Exp Tumor Res 1964;14:85 133.
67. Furst A. Bioassay of metals for carcinogenesis: whole animals. Envi ron Health Perspect 1981;40:83 91.
68. Vasiliev JM. Cell microenvironment and carcinogenesis in vivo and in vitro. IARC Sci Publ 1983;51:247 56.
69. Bischoff F, Bryson G. Carcinogenesis through solid state surfaces. Prog Exp Tumor Res 1964;14:85 133.
70. James SJ, Pogribna M, Miller BJ, Bolon B, Muskjelishvili L. Charac terization of cellular response to silicone implants in rats: implications for foreign body carcinogenesis. Biomaterials 1997;18:667 75.
71. Tazawa H, Tatemichi M, Sawa T, Gilibert I, Ma N, Hiraku Y, Done hower LA, Ohgaki H, Kawanishi S, Ohshima H. Oxidative and nitra tive stress caused by subcutaneous implantation of a foreign body accelerates sarcoma development in Trp53+/− mice. Carcinogenesis 2007;28:191 8.
72. Hussain SP, Amstad P, Raja K, Ambs S, Nagashima M, Bennett WP, Shields PG, Ham AJ, Swenberg JA, Marrogi AJ, Harris CC. Increased p53 mutation load in noncancerous colon tissue from ulcerative coli tis: a cancer-prone chronic inflammatory disease. Cancer Res 2000;60:3333 7.
73. Tatemichi M, Sawa T, Gilibert I, Tazawa H, Katoh T, Ohshima H. Increased risk of intestinal type of gastric adenocarcinoma in Japanese women associated with long forms of CCTTT pentanucleotide repeat in the inducible nitric oxide synthase promoter. Cancer Lett 2005; 217:197 202.
74. Ellie E, Loiseau H, Lafond F, Arsaut J, Demotes Mainard J. Differen tial expression of inducible nitric oxide synthase mRNA in human brain tumours. Neuroreport 1995;7:294 6.
75. Thomsen LL, Miles DW, Happerfield L, Bobrow LG, Knowles RG, Moncada S. Nitric oxide synthase activity in human breast cancer. Br J Cancer 1995;72:41 4.
76. Dogan AU, Baris YI, Emri S, Steele I, Elmishad AG, Carbone M. Genetic predisposition to fiber carcinogenesis causes a mesothelioma epidemic in Turkey. Cancer Res 2006;66:5063 8.
77. Heppner GH, Dorcey L. In: Heppner GH, Fulton AM, eds. Macro phages and cancer. Boca Raton, Florida: CRC Press, 1988.197 208.
78. Kobayashi H, Sendo F, Shirai T, Kaji H, Kodama T. Modification in growth of transplantable rat tumors exposed to Friend virus. J Natl Cancer Inst 1969;42:413 9.
79. Itaya T, Yamagiwa S, Okada F, Oikawa T, Kuzumaki N, Takeichi N, Hosokawa M, Kobayashi H. Xenogenization of a mouse lung carcinoma (3LL) by transfection with an allogeneic class I major histocompatibility complex gene (H $2L^d$). Cancer Res 1987;47: 3136 40.
80. Ishikawa M, Okada F, Hamada J I, Hosokawa M, Kobayashi H. Changes in the tumorigenic and metastatic properties of tumor cells treated with quercetin or 5 azacytidine. Int J Cancer 1987;39:338 42.
81. Hamada J, Takeichi N, Okada F, Ren J, Li X, Hosokawa M, Kobaya shi H. Progression of weakly malignant clone cells derived from rat mammary carcinoma by host cells reactive to plastic plates. Jpn J Cancer Res 1992;83:483 90.
82. Cristofanilli M, Buzdar AU, Hortobagyi GN. Update on the manage ment of inflammatory breast cancer. Oncologist 2003;8:141–8.
83. Li X, Nagayasu H, Hamada J I, Hosokawa M, Takeichi N. Enhance ment of tumorigenicity and invasion capacity of rat mammary adeno carcinoma cells by epidermal growth factor and transforming growth factor . Jpn J Cancer Res 1993;84:1145 9.
84. Hamada J, Nakata D, Nakae D, Kobayashi Y, Akai H, Konishi Y, Okada F, Shibata T, Hosokawa M, Moriuchi T. Increased oxidative DNA damage in mammary tumor cells by continuous epidermal growth factor stimulation. J Natl Cancer Inst 2001;93:214 9.
85. Nagayasu H, Hamada J, Nakata D, Shibata T, Kobayashi M, Hoso kawa M, Takeichi N. Reversible and irreversible tumor progression of a weakly malignant rat mammary carcinoma cell line by in vitro ex posure to epidermal growth factor. Int J Oncol 1998;12:197 202.
86. Okada F, Hosokawa M, Hasegawa J, Ishikawa M, Chiba I, Nakamura Y, Kobayashi H. Regression mechanisms of mouse fibrosarcoma cells after in vitro exposure to quercetin: diminution of tumorigenicity with a corresponding decrease in the production of prostaglandin E_2. Can cer Immunol Immunother 1990;31:358 64.
87. Okada F, Hosokawa M, Hamada J, Hasegawa J, Mizutani M, Takeichi N, Kobayashi H. Progression of a weakly tumorigenic mouse fibrosar- coma at the site of early phase of inflammation caused by plastic plates. Jpn J Cancer Res 1993;84:1230 6.

88. Okada F, Hosokawa M, Hamada J I, Hasegawa J, Kato M, Mizutani M, Ren J, Takeichi N, Kobayashi H. Malignant progression of a mouse fibrosarcoma by host cells reactive to a foreign body (gelatin sponge). Br J Cancer 1992;66:635 9.
89. Okada F, Fujii J. Molecular mechanisms of inflammation-induced carcinogenesis. J Clin Biochem Nutr 2006;39:103 13.
90. Marx J. Biomedicine. Thymosins: clinical promise after a decades long search. Science 2007;316:682 3.
91. Kobayashi T, Okada F, Fujii N, Tomita N, Ito S, Tazawa H, Aoyama T, Choi SK, Shibata T, Fujita H, Hosokawa M. Thymosin 4 regulates motility and metastasis of malignant mouse fibrosarcoma cells. Am J Pathol 2002;160:869 82.
92. Habelhah H, Okada F, Kobayashi M, Nakai K, Choi S, Hamada J, Moriuchi T, Kaya M, Yoshida K, Fujinaga K, Hosokawa M. Increased E1AF expression in mouse fibrosarcoma promotes metastasis through induction of MT1 MMP expression. Oncogene 1999;18:1771 6.
93. Okada F, Tazawa H, Kobayashi T, Kobayashi M, Hosokawa M. Involvement of reactive nitrogen oxides for acquisition of metastatic properties of benign tumors in a model of inflammation-based tumor progression. Nitric Oxide 2006;14:122 9.
94. Tazawa H, Okada F, Kobayashi T, Tada M, Mori Y, Une Y, Sendo F, Kobayashi M, Hosokawa M. Infiltration of neutrophils is required for acquisition of metastatic phenotype of benign murine fibrosarcoma cells: Implication of inflammation-associated carcinogenesis and tumor progression. Am J Pathol 2003;163:2221 32.
95. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. Proc Natl Acad Sci USA 2006;103:12493 8.
96. Okada F, Nakai K, Kobayashi T, Shibata T, Tagami S, Kawakami Y, Kitazawa T, Kominami R, Yoshimura S, Suzuki K, Taniguchi N, Inanami O, et al. Inflammatory-cell-mediated tumour progression and minisatellite mutation correlate with the decrease of antioxidative enzymes in murine fibrosarcoma cells. Br J Cancer 1999;79:377–85.
97. Babior BM. NADPH oxidase: an update. Blood 1999;93:1464 76.
98. Okada F, Kobayashi M, Tanaka H, Kobayashi T, Tazawa H, Iuchi Y, Onuma K, Hosokawa M, Dinauer MC, Hunt NH. The role of nicotinamide adenine dinucleotide phosphate oxidase derived reactive oxygen species in the acquisition of metastatic ability of tumor cells. Am J Pathol 2006;169:294 302.
99. Yoshida N, Granger DN, Evans DJ, Evans DG, Graham DY, Anderson DC, Wolf RE, Kvietys PR. Mechanisms involved in *Helicobacter pylori*-induced inflammation. Gastroenterology 1993;105:1431–40.
100. Parkin DM, Ohshima H, Srivatanakul P, Vatanasapt V. Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. Cancer Epidemiol Biomarkers Prev. 1993;2:537 44.
101. Levin B. Ulcerative colitis and colon cancer: biology and surveil lance. J Cell Biochem Suppl 1992;16G:47 50.
102. Choi PM, Zelig MP. Similarity of colorectal cancer in Crohn s disease and ulcerative colitis: implications for carcinogenesis and prevention. Gut 1994;35:950 4.
103. Kawaguchi T, Miyaki M, Masui T, Watanabe M, Ohta H, Maruyama M, Utakoji T, Kitagawa T. Establishment and characterization of epithelial cell line with quasi normal chromosomes from a tubular adenoma of a familial polyposis coli patient. Jpn J Cancer Res 1991;82:138 41.
104. Okada F, Kawaguchi T, Habelhah H, Kobayashi T, Tazawa H, Takeichi N, Kitagawa T, Hosokawa M. Conversion of human colonic adenoma cells to adenocarcinoma cells through inflammation in nude mice. Lab Invest 2000;80:1617 28.
105. Sanford KS, Boone CW, Merwin RM, Jones GM, Garrison CU. The plate implant as a bioassay for the neoplastic potential of cultured cells. Int J Cancer 1980;25:509 16.
106. Tatemichi M, Ogura T, Ishii Y, Esumi H. Role of inducible nitric oxide synthase activity in inflammation related carcinogenesis. Nitric Oxide 2002;6:473 4.
107. Fukuda T, Hirayama Y, Mitani H, Maeda H, Tsutsumi M, Konishi Y, Hino O. Generation of metastatic variants of Eker renal carcinoma cell lines for experimental investigation of renal cancer metastasis. Jpn J Cancer Res 1998;89:1104 8.
108. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860 7.
109. Habelhah H, Okada F, Nakai K, Choi SK, Hamada J, Kobayashi M, Hosokawa M. Polysaccharide K induces Mn superoxide dismutase (Mn SOD) in tumor tissues and inhibits malignant progression of QR 32 tumor cells: possible roles of interferon α, tumor necrosis factor α and transforming growth factor in Mn SOD induction by polysaccharide K. Cancer Immunol Immunother 1998;46:338 44.
110. Okada F, Shionoya H, Kobayashi M, Kobayashi T, Tazawa H, Onuma K, Iuchi Y, Matsubara N, Ijichi T, Dugas B, Hosokawa M. Prevention of inflammation-mediated acquisition of metastatic properties of benign mouse fibrosarcoma cells by administration of an orally available superoxide dismutase. Br J Cancer 2006;94:854 62.
111. Kawanishi S, Hiraku Y, Pinlaor S, Ma N. Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. Bio Chem 2006;387:365 72.
112. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276 85.

Exhibit 64

Free Radical Research, May 2010; 44(5): 479–496

informa
healthcare

Reactive oxygen species in cancer

GEOU-YARH LIOU & PETER STORZ

Department of Cancer Biology, Mayo Clinic, 4500 San Pablo Road, Jacksonville FL 32224, USA

(Received date: 26 August 2009; in revised form date: 28 January 2010)

Abstract

Elevated rates of reactive oxygen species (ROS) have been detected in almost all cancers, where they promote many aspects of tumour development and progression. However, tumour cells also express increased levels of antioxidant proteins to detoxify from ROS, suggesting that a delicate balance of intracellular ROS levels is required for cancer cell function. Further, the radical generated, the location of its generation, as well as the local concentration is important for the cellular functions of ROS in cancer. A challenge for novel therapeutic strategies will be the fine tuning of intracellular ROS signalling to effectively deprive cells from ROS-induced tumour promoting events, towards tipping the balance to ROS-induced apoptotic signalling. Alternatively, therapeutic antioxidants may prevent early events in tumour development, where ROS are important. However, to effectively target cancer cells specific ROS-sensing signalling pathways that mediate the diverse stress-regulated cellular functions need to be identified. This review discusses the generation of ROS within tumour cells, their detoxification, their cellular effects, as well as the major signalling cascades they utilize, but also provides an outlook on their modulation in therapeutics.

Keywords: *Oxidative stress, reactive oxygen species, cancer, signal transduction*

Abbreviations: *5-LOX, 5-Lipoxygenase; AP-1, activating protein-1; Ask-1, apoptosis signal-regulating kinase-1; BER, base excision repair; BITC, benzyl isothiocyanate; BPQ, benzo(a)pyrene quinines; CREB, cyclic AMP response element (CRE)-binding protein; CSC, cancer stem cell; ECM, extracellular matrix; EGCG, epigallocate-3-gallate; EGF, epidermal growth factor; EMT, epithelial-to-mesenchymal transition; Erk1/2, extracellular-regulated kinase 1/2; Ets, E twenty-six; FAK, focal adhesion kinase; FGF, fibroblast growth factor; GCS, glutamylcysteine synthetase; GPX, glutathione peroxidase; GSH, glutathione; GSSG, glutathione disulphide; GST, glutathione S-transferase; HIF-1, hypoxia inducible factor-1; ICAM-1, intracellular adhesion protein 1; IFNγ, interferon γ; IKK, IκB kinase; IL, interleukin; IOA, isoobtusilactone A; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase kinase; MKP3, mitogen-activated protein kinase phosphatase 3; MMP, matrix metalloproteinase; NAC, N-acetyl-L-cysteine; NER, nuclear excision repair; NF-κB, nuclear factor κ-B; NIK, NF-κB-inducing kinase; PDGF, platelet-derived growth factor; PDK-1, 3'-phosphoinositide-dependent kinase-1; PDTC, pyrrolidine dithiocarbamate; PI3K, phosphoinositide 3-kinase; PKB, protein kinase B; PKC, protein kinase C; PKD, protein kinase D; Prx, peroxiredoxin; PST, pancratistatin; PTEN, phosphatase and tensin homologue deleted on chromosome 10; ROS, reactive oxygen species; SAL, salvicine; SOD, superoxide dismutase; TGFβ, transforming growth factor β; TIMP, tissue inhibitor of metalloproteinase; TNFα, tumour necrosis factor α; TPL, triphala; TRAF, TNF receptor-associated factor; VEGF, vesicular epithelial growth factor.*

Reactive oxygen species

Reactive oxygen species are radicals, ions or molecules that have a single unpaired electron in their outermost shell of electrons. Due to this character, ROS are highly reactive. ROS can be categorized into two groups: free oxygen radicals and non-radical ROS. Free oxygen radicals include superoxide ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$), nitric oxide ($NO^{\bullet}$), organic radicals ($R^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$), thiyl radicals ($RS^{\bullet}$), sulphonyl radicals ($ROS^{\bullet}$), thiyl peroxyl

Correspondence: Peter Storz, Department of Cancer Biology, Mayo Clinic, Griffin Rm 306, 4500 San Pablo Road, Jacksonville FL 32224, USA. Tel: 904 953-6909. Fax: 904 953-0277. Email: storz.peter@mayo.edu

ISSN 1071-5762 print/ISSN 1029-2470 online © 2010 Informa UK Ltd. (Informa Healthcare, Taylor & Francis AS)
DOI: 10.3109/10715761003667554

radicals (RSOO) and disulphides (RSSR). Non-radical ROS include hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone/trioxygen (O_3), organic hydroperoxides (ROOH), hypochloride (HOCl), peroxynitrite (ONO), nitrosoperoxycarbonate anion (O NOOCO$_2$), nitrocarbonate anion (O_2NOCO_2), dinitrogen dioxide (N_2O_2), nitronium (NO_2) and highly reactive lipid- or carbohydrate-derived carbonyl compounds. Among them, superoxide, hydrogen peroxide and hydroxyl radicals are the most well studied ROS in cancer.

Cellular sources for ROS

In cancer cells high levels of reactive oxygen species can result from increased metabolic activity, mitochondrial dysfunction, peroxisome activity, increased cellular receptor signalling, oncogene activity, increased activity of oxidases, cyclooxygenases, lipoxigenases and thymidine phosphorylase or through cross-talk with infiltrating immune cells [1–3].

In mitochondria, ROS are produced as an inevitable byproduct of oxidative phosphorylation (Figure 1). The electron transport chain encompasses complexes I IV and ATP synthase on the mitochondrial inner membrane. Superoxide is generated at complexes I and III and released into the inter-membrane space (~80% of the generated superoxide) or the mitochondrial matrix (~20%) [4]. The mitochondrial permeability transition pore (MPTP) in the outer membrane of the mitochondrion allows the leakage of superoxide into the cytoplasm ([5] and [6] for a more detailed description of mitochondrial ROS generation). Superoxide is dismutated to H_2O_2, either in the mitochondrial matrix (by MnSOD) or in the cytosol (by Cu/ZnSOD). H_2O_2 is a *bona fide* second messenger that is highly diffusible. Recent data suggest that hydrogen peroxide may cross cellular membranes through specific members of the aquaporin family [7]. For example, aquaporin-8 was detected in the inner mitochondrial membrane and suggested to function as a channel for water and potentially H_2O_2 [8]. In addition to the mitochondria, peroxisomes are other major sites of cellular ROS generation [9]. In these respiratory organelles, superoxide and H_2O_2 are generated through xanthine oxidase in the peroxisomal matrix and the peroxisomal membranes ([10,11], see [12] for a detailed review on ROS in peroxisomes).

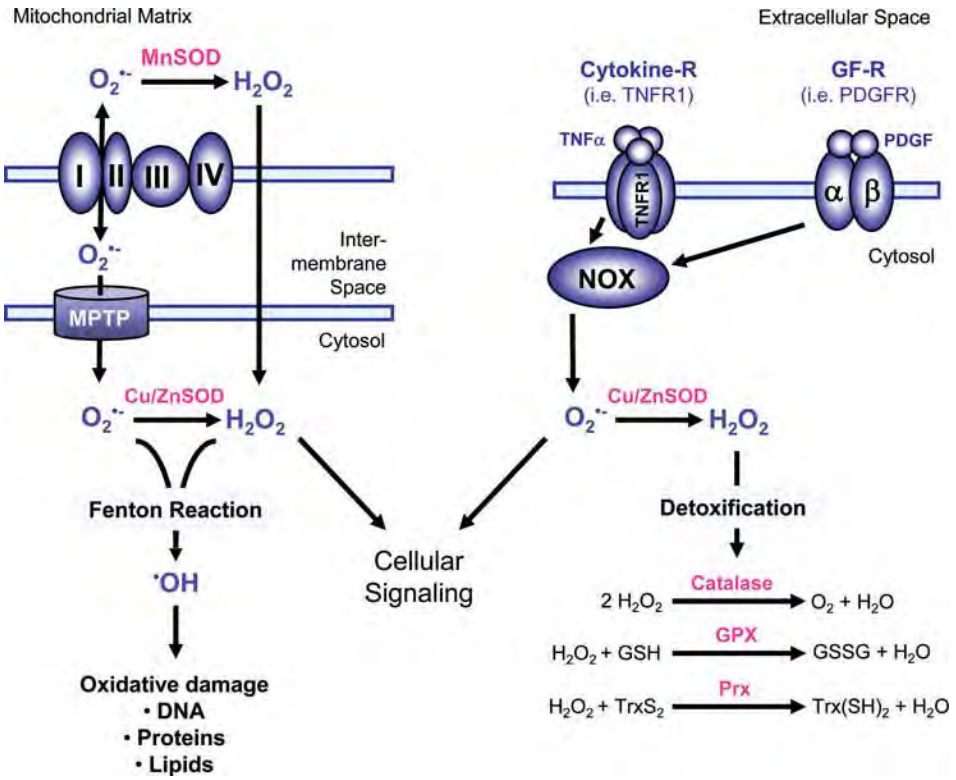

Figure 1. Major mechanisms of ROS generation and detoxification. Superoxide (O_2) radicals are generated at the inner membrane of the mitochondria as a byproduct of the electron transport chain and then release into the mitochondrial matrix or the cytosol via the mitochondrial permeability transition pore (MPTP). Superoxide is also generated through activation of NADPH oxidases (NOX), for example in response to growth factor receptor (GF-R) or cytokine receptor activation. SOD enzymes, such as MnSOD in the mitochondrial matrix or Cu/ZnSOD in the cytosol, reduce superoxide to H_2O_2. Several cytosolic antioxidant systems, including catalase, glutathione peroxidase (GPX) and peroxiredoxins (Prx), detoxify cells from hydrogen peroxide by reducing it to water. Both hydrogen peroxide and superoxide contribute to cellular signalling but also can form hydroxyl radicals (OH). Hydroxyl radicals are generated from O_2 and H_2O_2 in the Fenton reaction and have damaging functions for proteins, DNA and lipids.

Growth factors and cytokines stimulate the production of ROS to exert their diverse biological effects in cancer [13 16]. For example, an elevation of hydrogen peroxide and nitrite oxide levels was detected in tumour cells in response to interferon γ (IFNγ) and TNFα [17,18]. Further, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, transforming growth factor β (TGFβ), interleukin-1 (IL-1), tumour necrosis factor α (TNFα), angiotensin and lysophosphatidic acid all induce the formation of superoxide [13,16,19 23]. Activation of the small GTPase K-ras downstream of growth factors or its oncogenic mutation has been tightly associated with increased generation of superoxide and the incidence of various cancers [24 26]. Dependent on the cellular system, growth factors and mutant K-ras elevate intracellular superoxide levels through NADPH oxidase or mitochondria [1]. NADPH oxidase can also be activated via the RhoGTPase Rac-1 [27]. Rac-1-mediated generation of superoxide is induced by cell surface receptors including c-Met [28]. Active Rac-1 further was implicated to induce 5-Lipoxygenase (5-LOX)-mediated generation of H_2O_2 [29].

Many cancers arise from sites of chronic irritation, infection or inflammation. Recent data have expanded the concept that inflammation is a critical component of tumour progression [30 32]. Macrophages induce the generation of ROS within tumour cells through secretion of various stimuli, such as TNFα [1]. Production of ROS by neutrophils and macrophages as a mechanism to kill tumour cells is well established. In these cells, a rapid burst of superoxide formation primarily mediated by NAPDH oxidase leads to subsequent production of hydrogen peroxide [33,34]. Furthermore, during inflammation processes, activated macrophages also generate nitric oxide which reacts with superoxide to produce peroxinitrite radicals that are similar in their activity to hydroxyl radicals and contribute to tumour cell apoptosis [35].

Cellular detoxification from ROS

Under normal physiological conditions, the intracellular levels of ROS are steadily maintained to prevent cells from damage. Detoxification from ROS is facilitated by non-enzymatic molecules (i.e. glutathione, flavenoids and vitamins A, C and E) or through antioxidant enzymes which specifically scavenge different kinds of ROS (Figure 1).

Superoxide dismutases (SODs) are metalloenzymes which catalyse the dismutation of superoxide anion to oxygen and hydrogen peroxide. They ubiquitously exist in eukaryotes and prokaryotes. Superoxide dismutases utilize metal ions such as copper (Cu^2), zinc (Zn^2), manganese (Mn^2) or iron (Fe^2) as cofactors. The different SOD enzymes are located in different compartments of the cell and are highly specific in regulating linked biological processes [36].

Catalase facilitates the decomposition of hydrogen peroxide to water and oxygen. The major localization of catalase in most eukaryotes is in the cytosol and peroxisomes [37 39]. Peroxiredoxins are thioredoxin peroxidases that catalyse the reduction of hydrogen peroxide, organic hydroperoxides and peroxynitrite [40 42]. They are divided into three classes: typical 2-cysteine peroxiredoxins (PrxI-IV), atypical 2-cysteine peroxiredoxins (PrxV) and 1-cysteine peroxiredoxins (PrxVI). Interestingly, PrxI knockout mice show increased levels of oxidative stress and die prematurely of cancer [43]. The thioredoxin system consists of thioredoxin and thioredoxin reductase. The catalytic site of thioredoxin contains two neighbouring cysteines which are cycled between an active (reduced) dithiol form and an oxidized disulphide form [44]. In its active state, thioredoxin scavenges reactive oxygen species and keeps proteins in their reduced state [45]. Thioredoxin is regenerated by thioredoxin reductases which utilize NADPH as an electron donor [46].

The glutathione system includes glutathione (GSH), glutathione reductase, glutathione peroxidases (GPX) and glutathione S-transferases (GST). Glutathione protects cells from oxidative stress by reducing disulphide bonds of cytoplasmic proteins to cysteines. During this process, glutathione is oxidized to glutathione disulphide (GSSG). Glutathione peroxidases (GPX) catalyse the breakdown of hydrogen peroxide and organic hydroperoxides [47,48]. Glutathione reductase reduces GSSG and refills GSH pools [49]. Under physiological conditions, glutathione almost exclusively exists in its reduced form because of a constitutive activity of glutathione reductase in cells [50]. Glutathione S-transferases are detoxification enzymes that catalyse the conjunction of GSH to a variety of exogenous and endogenous electrophilic compounds [51 53]. GSTs are over-expressed in a wide variety of tumours to regulate MAPK pathways and are also involved in the development of resistance to chemotherapeutics [51].

Signalling pathways regulated by ROS in cancer

ROS-sensitive signalling pathways are persistently elevated in many types of cancers, where they participate in cell growth/proliferation, differentiation, protein synthesis, glucose metabolism, cell survival and inflammation [1]. Reactive oxygen species, particularly hydrogen peroxide, can act as second messengers in cellular signalling [16,54 57]. H_2O_2 regulates protein activity through reversible oxidation of its targets including protein tyrosine phosphatases, protein tyrosine kinases, receptor tyrosine kinases and transcription factors [1,27,58]. In the following paragraphs, we focus on ROS-mediated regulation of the mitogen-activated protein (MAP) kinase/Erk cascade,

phosphoinositide-3-kinase (PI3K)/Akt-regulated signalling cascades, as well as the IκB kinase (IKK)/nuclear factor κ-B (NF-κB)-activating pathways (Figure 2). Other ROS-regulated signalling pathways are included later.

ROS-mediated regulation of the MAPK/Erk1/2 pathway

The activation of the MAPK (mitogen-activated protein kinase)/Erk1/2 (extracellular-regulated kinase 1/2) pathway in cancer is mediated through growth factors and K-ras and was functionally linked to increased cell proliferation [59,60]. For instance, in human breast cancer cells, Erk1/2 activated by hydrogen peroxide generated as a byproduct during oestrogen metabolism increases cell proliferation [61,62]. Several mechanisms of how ROS activate Erk1/2 are known. For example Ras, which is an upstream activator for Erk1/2, can be activated directly through oxidative modification at its cysteine 118 residue, leading to the inhibition of GDP/GTP exchange [63]. ROS also activate upstream kinases of Erk1/2 such as $p90^{RSK}$ [64,65]. It was recently shown that increased Erk1/2 activity in ovarian cancer cells in the presence of the high concentration of endogenous ROS results from sustained ubiquitination and loss of endogenous MKP3 (mitogen-activated protein kinase phosphatase 3), a phosphatase that negatively-regulates Erk1/2 activity [64,65]. Additionally to its effects on cell proliferation, it was also shown in multiple cancers (i.e. ovarian cancer, breast cancer, melanoma and leukaemia) that the activation of Erk1/2 through ROS increases cell survival, anchorage-independent growth and motility [60,65,66].

While a role for ROS-activated Erk1/2 signalling in cell proliferation is well established [61,65,67], its ability to regulate cancer cell survival seems to be cell type specific [64,68,69]. For example, treatment of MCF-7 and MDA-MB-435 breast cancer cells with ROS scavengers or inhibitors that target Erk1/2 or its upstream kinase MEK (mitogen-activated protein kinase kinase) promote apoptosis and cell adhesion [70,71]. In an animal model for skin cancer, murine keratinocytes lacking Tiam1, an upstream activator of Erk1/2, show low levels of intracellular ROS [69]. These keratinocytes are more sensitized to apoptosis upon deprivation of EGF and insulin, implicating that Erk1/2 activation though Tiam1 and ROS is required for cell survival of skin cancer [69]. In contrast, in human pancreatic cancer and glioma cells, activation of Erk1/2 upon treatment with exogenous H_2O_2 triggers cell death and this probably is due to the high basal level of ROS in these cancer cells [72 76]. In line with

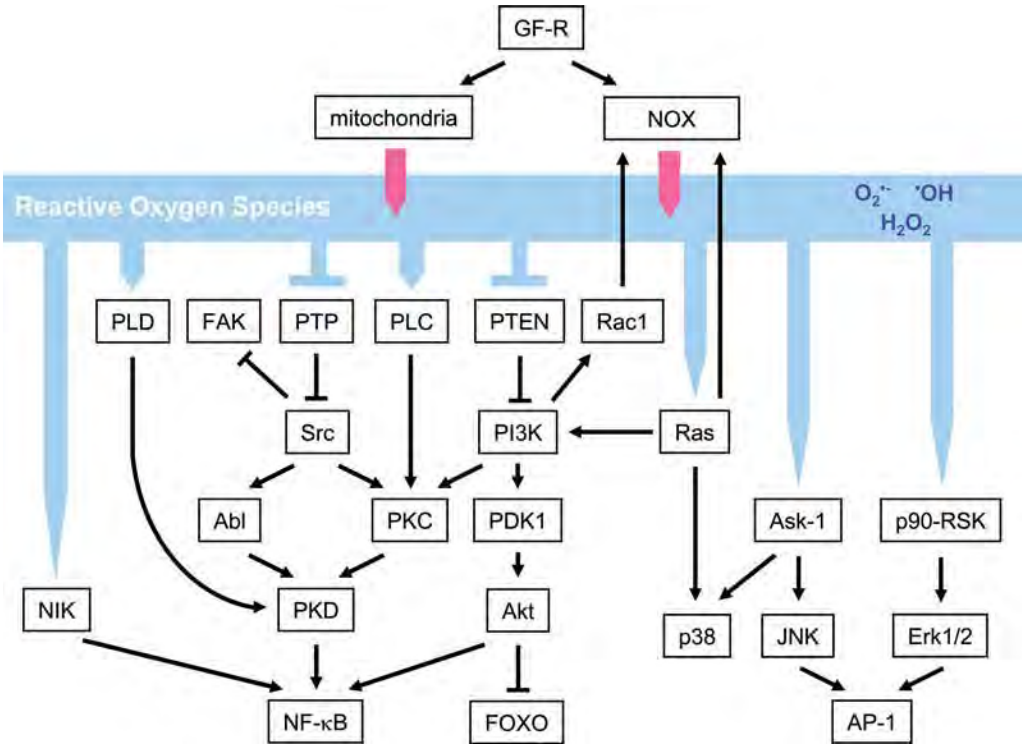

Figure 2. ROS-induced cellular signalling. Reactive oxygen species in cells can be generated by growth factor signalling through activation of the NADPH oxidase NOX1 or through the mitochondria. These ROS then can induce cellular signalling cascades by reversible oxidation of phosphatases such as PTEN or PTP in their active site cysteins or by direct oxidation of kinases such as Src. This leads to the activation of several signalling cascades such as a Src/PKD1-dependent NF-κB activation mechanism, the MAPK (Erk1/2, p38 and JNK) signalling cascades, as well as the PI3K/Akt signalling pathway. Other mechanisms, by which ROS induce cellular signalling is through activation of redox-regulated transcription factors such as AP-1 or FOXO.

these *in vitro* data is an *in vivo* study showning that ROS-mediated increase of Erk1/2 activation loop phosphorylation suppresses the growth of pancreatic tumour cell xenografts [77].

Oxidative stress regulation of the PI3K/Akt pathway

Akt (or protein kinase B; PKB) mediates cell survival through phosphorylation and inactivation of its substrates such as the pro-apoptotic proteins Bad, Bax, Bim or FOXO transcription factors [78 83]. In breast cancer, ROS generation during oestrogen metabolism or other potential mammary carcinogens was shown to activate the PI3K/Akt signalling pathway [84,85]. Hydrogen peroxide generated by epithelial growth factor (EGF) in human ovarian cancer cells activates Akt and p70 S6K1, a substrate of Akt that regulates protein synthesis [86]. Moreover, the inhibition of ROS in the human pancreatic tumour cell line Panc-1 reduced the levels of phosphorylated (active) Akt and induced apoptosis [87]. Akt activity is tightly controlled by a signalling cascade that encompasses the kinases PDK-1 (3 -phosphoinositide-dependent kinase-1), mTOR and PI3K as well as the phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10). PDK-1 and mTOR regulate Akt activating phosphorylations at S473 and T308, whereas PI3K generates phosphatidylinositol-3,4,5-triphosphate (PIP_3), which serves as a membrane anchor [88]. PTEN negatively regulates PIP_3 levels and thus decreases Akt activity [89,90]. Treating cells with exogenous hydrogen peroxide it was shown that Akt and PDK-1 can be activated by oxidative stress [91,92]. This correlates with the observation that PTEN is reversibly inactivated by H_2O_2 [93]. Loss of PTEN increases basal levels of hydrogen peroxide and superoxide due to depletion of the expression of several antioxidant enzymes including peroxiredoxins and copper/zinc superoxide dismutase [94]. This suggests a constant activation of Akt through enhanced ROS production due to PTEN ablation, but also oxidative stress-mediated activation of its upstream kinases.

ROS regulation of the IKK/NF-κB pathway

In many cancers the transcription factor NF-κB is uncoupled from its normal modes of regulation and shows increased activity [95 98]. Recent studies have established a crucial role for NF-κB in tumour cell survival, regulation of cell cycle and proliferation, cellular adhesion and development of drug resistance in cancer cells during therapy [99 101].

NF-κB is a redox-regulated sensor for oxidative stress [102] and is activated by low doses of hydrogen peroxide [103]. When inactive, NF-κB is tightly bound to its inhibitor IκB that sequesters the transcription factor in the cytosol [104 108]. The canonical activation of NF-κB is mediated through the NF-κB-inducing kinase (NIK) and the IκB kinase (IKK) complex, consisting of IKKα, IKKβ and NEMO. Upon its activation through cytokines such as TNFα or IL-1, NIK phosphorylates and activates its downstream targets, the kinases IKKα and IKKβ [104,109 111]. Active IKKs phosphorylate IκB and this leads to its subsequent ubiquitination and proteosomal degradation [112,113]. Degradation of IκB translocates NF-κB to the nucleus, where it acts as a transcription factor to induce the expression of anti-apoptotic and anti-inflammatory genes [114].

Oxidative stress activates NF-κB through a variety of distinct signalling pathways [115]. For example, treatment of MCF-7 breast cancer cells with TNFα, IL-1β or the mammary carcinogen sodium arsenite generates hydrogen peroxide and superoxide, which translates to the activation of NF-κB and increased cell proliferation [116 118]. In oral squamous carcinoma cells silencing of the antioxidant superoxide dismutase (SOD) increased basal ROS levels correlating with increased NIK and NF-κB activity [119]. The mechanism of how ROS activates NIK is most likely via oxidative inhibition of regulatory phosphatases [116]. Recent work from our group delineated an IKK-dependent NF-κB-inducing signalling pathway that is activated by increased cellular oxidative stress, induced either by exogenous treatment of cells with hydrogen peroxide, by rotenone-mediated mitochondrial generation of superoxide or inhibition of intracellular antioxidant systems such as the glutathione system [120,121]. In this pathway, NF-κB is activated through the lipase PLD1 and the kinases Src, Abl and Protein Kinase Cδ (PKCδ), whose signalling converge at the level of Protein Kinase D1 (PKD1) [120,122 124]. PKD1 is upstream of the IKK complex and mediates the activation of NF-κB through IKKβ [121]. In addition to this, IKK-independent activation of NF-κB in response to ROS can occur through tyrosine phosphorylation of IκBα, leading to a release from the IKK complex, but not to its degradation [125,126].

Specific functions of ROS in cancer

Oxidative stress-mediated signalling events have been reported to affect all characters of cancer cell behaviour [1,2,127]. For instance, ROS in cancer are involved in cell cycle progression and proliferation, cell survival and apoptosis, energy metabolism, cell morphology, cell cell adhesion, cell motility, angiogenesis and maintenance of tumour stemness (Figure 3).

ROS in tumour cell proliferation

Low doses of hydrogen peroxide and superoxide stimulate cell proliferation in a wide variety of cancer cell

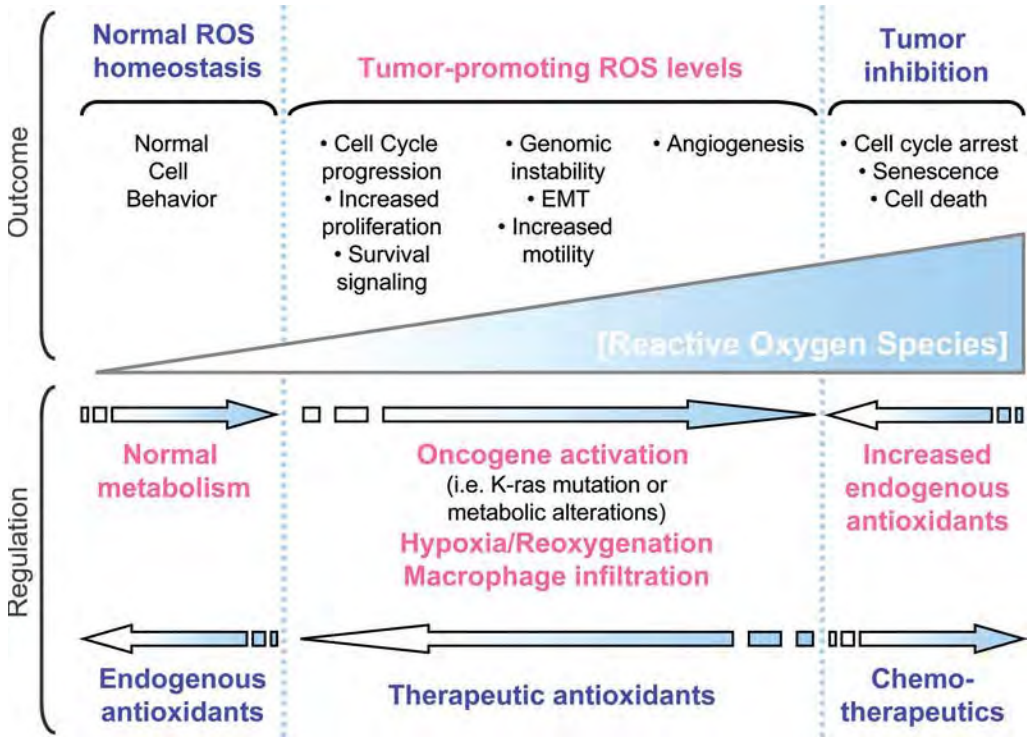

Figure 3. Generation, regulation and effects of cellular ROS. ROS are generated in normal cellular processes and cells express antioxidants to deplete intracellular levels of oxygen radicals. Tumourigenic events including oncogene activation (i.e. mutation of K-ras), metabolic alterations or macrophage infiltration or hypoxia/reoxygenation processes in tissues can increase intracellular ROS levels and promote tumour formation or progression. These tumour-promoting ROS levels can lead to cell cycle progression, increased proliferation and survival signalling, EMT, increased motility, genomic instability and increased angiogenesis and may be negatively-regulated by therapeutic antioxidants. Finally, excessive increase in intracellular ROS levels as mediated by chemotherapeutics, can induce cell cycle arrest, senescence or cell death of tumour cells, but may be repulsed by the tumour cells through an increase in the expression of endogenous antioxidants.

types [1,128]. For example, intracellular oxidative stress in breast cancer cells is increased through the translocation of oestrogen to the mitochondria [62, 129 131]. Mitochondria-derived ROS regulate both cell proliferation and quiescence. This is mediated by MnSOD activity which serves as a mitochondrial ROS switch [132]. Decreased MnSOD activity favours proliferation, due to increased superoxide and low hydrogen peroxide levels, while increasing MnSOD activity drives the proliferating cells to transit into quiescence, due to increased generation of hydrogen peroxide [133]. In breast cancer cells, inhibition of the mitochondrial uniporter blocks ROS generation and suppresses oestrogen-induced cell proliferation, suggesting a role of mitochondrial ROS in tumour growth [134]. Oestrogen-induced cell proliferation results from ROS-mediated activation of the Erk1/2 MAPK signalling pathway and the transcription factor CREB (cyclic AMP response element (CRE)-binding protein) [61,131].

Reactive oxygen species can upregulate the mRNA levels of cyclins that participate in the cell cycle to expedite G1 to S phase transition, including cyclin B2, cyclin D3, cyclin E1 and cyclin E2 [130]. It was shown that loss of the redox control of the cell cycle in normal MCF-10A cells may contribute to aberrant proliferation [135]. The treatment of MCF-10A cells with the antioxidant NAC caused delays in the progression from G1 to S accompanied with a decrease in cyclin D1 levels [135]. Further, the environmental carcinogen sodium arsenite stimulates ROS production in breast cancer cells and potentiates S phase progression and subsequent cell proliferation [118]. Likewise, benzo(a)pyrene quinines (BPQs) imitate growth factor signalling and increase mammary epithelial cell growth rates through induction of superoxide and hydrogen peroxide [84].

Conversely, antioxidants inhibit tumour cell proliferation [136]. For example, pancreatic cancer cell lines generally show high basal levels of endogenous oxidative stress as compared to normal cells [1]. These increased ROS levels have been linked to increased proliferation. A stable ectopic expression of the highly-active antioxidant enzyme MnSOD reduces the cell growth rate of pancreatic tumour cells [72]. Moreover, the expression levels and activities of endogenous MnSOD, Cu/ZnSOD, catalase and glutathione peroxidase reversely correlate with cell doubling times in various pancreatic cancer cell lines [72,73]. ATM (ataxia telangiectasia mutated) is one of the proteins involved in cell cycle regulation that are activated by ROS. Patients lacking ATM show higher levels of oxidative

damage and similar effects, obtained with ATM knockout mice can be rescued with administration of antioxidants [137,138]. Altogether, this suggests ROS as positive regulators of tumour cell proliferation by modulating key proteins in cell cycle progression.

ROS in apoptosis and cell survival

A disproportional increase in intracellular ROS can induce cancer cell cycle arrest, senescence and apoptosis. This can be achieved with cancer chemotherapy, depletion of cells from antioxidant proteins or generation of ROS by immune cells. Apoptosis is linked to an increase in mitochondrial oxidative stress that causes cytochrome *c* release, an unrevocable event that leads to the activation of caspases and cell death [139,140]. Additionally, superoxide generation through the Rac-1/NADPH oxidase pathway can also induce pro-apoptotic signalling [141].

Mitochondrial release of H_2O_2 and NO upon apoptotic signals leads to the activation of c-Jun N-terminal kinases (JNKs) [139,142]. In response to ROS, JNKs catalyse the phosphorylation and down-regulation of anti-apoptotic proteins such as Bcl-2 and Bcl-XL [139]. Both Bcl-2 and Bcl-XL have been shown to antagonize ROS generation and to protect cells from ROS-mediated apoptosis [143,144]. JNK also alters the composition of the Bax/Bcl-2 complex by increasing the expression of Bax, leading to formation of Bax homodimers, resulting in dissipation of mitochondrial membrane integrity [145 148].

p38, another MAPK family member, was also implicated in apoptotic signalling in response to increased generation of ROS. Both p38 and JNK are activated through Ask-1 (apoptosis signal-regulating kinase-1), whose activity is regulated by its interaction with thioredoxin. Thioredoxin is a redox-regulated protein that in its reduced form binds and inhibits Ask-1 [149, 150]. In addition to Ask-1-induced signalling cascades, other signalling proteins such as forkhead transcription factors (i.e. FOXO3a), p66Shc and p53 have been implicated in the induction of apoptosis in response to ROS [78,151]. For example, an interesting hypothesis is that constitutive oxidative stress in tumour cells may lead to the selection of p53-deficient clones that are resistant to apoptosis [1].

Death receptors such as the TNF receptor I mainly induce ROS generation via the mitochondria, leading to caspase activation and cell death [152]. However, TRAF4 (TNF receptor-associated factor4), a component of the TNFα signalling pathway, also binds to the NADPH oxidase complex to activate JNK [153], suggesting that death receptors may use several ways to induce ROS within cells. Notably, TNF-induced oxidative stress also mediates anti-apoptotic signalling by inducing the expression of MnSOD and catalase through NF-κB [154].

In the above signalling events high levels of ROS turn on cell death signalling. However, it recently became clear that low levels of oxidative stress can also actively promote cell survival signalling. Such a ROS-mediated survival pathway is regulated by protein kinase D1 (PKD1) [120,121,124,155 157]. Elevation of intracellular mitochondrial ROS levels activates PKD1 and subsequently NF-κB, leading to upregulation of antioxidant proteins such as MnSOD and anti-apoptotic proteins such as A20 and cIAPs [158]. In this pathway PKD1 is activated through the tyrosine kinase Src. Src directly phosphorylates PKD1, but also facilitates further activating phosphorylations through the kinases PKCδ (a member of the novel PKC family) and Abl [6,120,121,123,124,142]. The elimination of this pathway sensitizes tumour cells to oxidative stress and increases their susceptibility to ROS-mediated cell death [155 157,159,160].

Another anti-apoptotic protein that is activated by ROS in cancer is Akt, a serine/threonine kinase that fosters cell survival through phosphorylation and inactivation of its pro-apoptotic substrates [78 83]. Akt activity is induced by multiple receptor tyrosine kinases such as PDGF-R as well as constitutively-active K-ras via activation of PI3K.

ROS as regulators of cell motility and metastasis

The treatment of carcinoma cells with hydrogen peroxide prior to intravenous injection into mice enhanced metastasis [161]. Additionally, sub-populations of the low- or non-motile breast cancer cell line MCF-7 that possess higher levels of endogenous ROS than the parental cells showed increased motility, and orthotopic tumours generated with these cell lines metastasized to lung, liver and spleen [162]. Furthermore, metastatic breast cancer and highly-invasive pancreatic cancer cells show lower levels and activities of the antioxidant enzyme MnSOD [73,163,164]. This illustrates that the intracellular redox state governs crucial steps for the metastatic process. This comprises decreased cell adhesion to the extracellular matrix, anchorage-independent survival, increased migratory and invasive potential, as well as intravasation.

Cell adhesion and migration are dependent on integrin binding to the extracellular matrix. Integrins elevate oxidant levels mainly by increasing cyclooxygenase-2 [165], but also through 5-lipoxygenases (5-LOX) and mitochondria [27,166]. In this context, an increase in mitochondrial ROS was linked to a first cellular contact with ECM and increases in cytosolic ROS were shown to contribute to cytoskeleton remodelling and actin stress fibre formation during a later phase of the process [27,167]. Targets for mitochondrial ROS in these processes are SHP-2 and FAK (focal adhesion kinase), while cytosolic ROS target the phosphatases LMW-PTP and SHP-2, receptor tyrosine kinases, Src-family kinases, FAK

and structural proteins such as β-actin (in more detail reviewed in [27]). Activation of phosphatases and Src occurs through direct oxidation, whereas activation of FAK is probably indirect through upstream signalling events leading to its tyrosine phosphorylation [168]. Both Src and FAK are initiators of focal adhesion formation in adherent cells, contributing to cell spreading, cell migration and prevention of cell death by anoikis.

Non-transformed cells require an anchorage to extracellular matrix (ECM) to execute the mitotic programme. In this process ROS act as key second messengers to facilitate proper mitosis [27,169]. A synergistic signalling between growth factors (GF) and integrins leads to an oxidative burst through a Rac-1-dependent increase in mitochondrial ROS [13,170]. This leads to oxidative inhibition of PTPs, activation of Src and other protein tyrosine kinases or structural proteins, with the net effect of increasing cell adhesion to ECM, cell spreading and proliferation.

Loss of cell-to-matrix adhesion in non-transformed cells triggers anoikis, a specific type of apoptosis. In contrast to non-transformed cells, tumour cells are protected from this process and show increased cell proliferation and independence of anchorage. Such resistance to anoikis allows tumour cells to survive outside their normal environment and to metastasize and form new colonies at distant sites. The mechanism of how tumour cells become independent of cell attachment signals is most likely through increased generation of intracellular ROS. Such increase in oxidative stress seems to mimic autocrine/adhesive signals, which in normal cells are mediated by growth factor and integrin signalling. For example, in prostate cancer cells redox-regulated anoikis resistance is mediated via Src and the EGF receptor [171]. Subsequently, this results in a constitutive deregulation of mitogenic pathways and proliferation independent of anchorage. It further allows cancer cells to abolish anoikis signals and escape apoptotic responses after a loss of cell/ECM contacts (for an excellent review on this topic see [27]).

Before cells migrate to distal sites, they undergo epithelial-mesenchymal transition (EMT) to release themselves from the restrain of the basal membrane. During this process, metalloproteinases (MMPs) are upregulated to degrade the proteins that compose the basal membrane. Treatment of murine mammary epithelial cells with MMP-3, a stromal protease that is upregulated in mammary tumours, increased their intracellular ROS levels (mainly H_2O_2) and led to EMT through induction of Rac1b RhoGTPase [172]. Moreover, application of NAC (*N*-acetyl-L-cysteine) to remove ROS abolished MMP-3-induced EMT [172], bolstering that MMP induces oxidative stress to lead to malignant transformation. This increase in ROS mediates oxidative damage to DNA and genomic instability. It further stimulates the expression of Snail, which previously was identified as one of the key-transcription factors regulating EMT. Other ROS-regulated genes relevant to EMT are E-cadherin, integrins and MMPs [173].

Activation of Rac and subsequent generation of ROS leads to NF-κB activation and MMP-1 production in response to integrin-mediated cell shape changes [170]. Rac-1 mediated changes in cellular ROS levels also increase the migratory potential of MCF-7 and T47D breast cancer cells, probably through NF-κB [174]. Similarly, Rac-1 is a downstream target for c-Met and Rac-1-mediated ROS generation was involved in Met s prometastatic signalling [28]. Moreover, Rac-1 has important functions in ROS mediated actin reorganization of migrating tumour cells [175]. Multiple processes regulate actin reorganization at the leading edge of migrating cells including the actin-severing protein cofilin [176,177]. Rac-1 activates NADPH oxidase (NOX) and ROS generated by this enzyme have been shown to activate the cofilin pathway and thus contribute to increased cell migration [177,178]. The tyrosine kinase Src also regulates NADPH oxidase 1 (NOX1) induced generation of ROS [179]. NOX1 is capable of transforming cells and is also required to maintain the transformed state [87,174]. NOX1-mediated ROS generation has been shown to be necessary for the formation of invadopodia, actin cytoskeleton-based structures that tumour cells use to invade [180].

Matrix metalloproteinases facilitate the degradation and reorganization of the extracellular matrix and their increased activation was associated with primary tumour growth, angiogenesis, increased tumour cell invasion, blood vessel penetration and metastasis [181 184]. ROS regulate not only the expression of MMPs, but also the inactivation of their inhibitors TIMP (tissue inhibitor of metalloproteinase) [185,186]. An important step in oxidative stress-mediated expression of MMP genes is the dismutation of mitochondrially-generated superoxide to hydrogen peroxide [187]. Hydrogen peroxide then regulates the expression of MMPs through activation of the Ras-Erk1/2-Ets (E twenty-six), Rac-1-JNK-AP-1 (activating protein-1) or p38 signalling pathways [188] (for a review on this topic see [184]). Further, the redox-sensitive transcription factors NF-κB and FOXO3a have been described as regulators of MMP expression [1,159]. Additionally to regulating MMP expression, ROS also can lead to the direct activation of MMPs through reactions with thiol groups in their catalytic domain [189].

Finally, ROS may also promote tumour cell metastasis by increasing the vascular permeability [181]. Increased activity of Rac-1 in primary endothelial cells mediates a loss of cell cell adhesions and loosens the integrity of the endothelium, which allows the intravasation of cancer cells [190]. It was shown that reverse (basolateral-to-apical) transendothelial migration

(TEM) of human melanoma cells is induced by hydrogen peroxide and can be blocked by thioredoxin [191]. Oxidative stress also regulates the expression of interleukin-8 (IL-8) and the cell surface protein ICAM-1 (intracellular adhesion protein 1, CD54) through NF-κB. Both ICAM-1 and IL-8 can regulate the trans-endothelial migration of tumour cells [192]. Further, phosphorylation of the heatshock protein Hsp27 by ROS-activated p38 induces changes in actin dynamics in vascular endothelial cells, which may contribute to facilitate invasive processes [193].

Hypoxia as a factor leading to tumour progression

Within a growing tumour mass cancer cells repeatedly face cycles of hypoxia and reoxygenation [194 196]. Limitations in oxygen supply due to prolonged hypoxia can result in cell death. Tumour cells can use the Warburg effect , a metabolic switch to glycolysis, to adapt to low oxygen tension [197]. Normal and tumour cells differ significantly in energy metabolism. Glucose is the primary energy source for normal cells. Normal cells switch to anaerobic glycolysis only when adequate oxygen supply is not available and mitochondrial function is suppressed [198]. A shift from aerobic to anaerobic metabolism in tumour cells occurs even under conditions of normoxia or after mitochondrial dysfunction, oncogenic transformation or loss of tumour suppressor genes [196,199].

The adaption of tumour cells to hypoxia contributes to the malignant phenotype and to aggressive tumour progression [200]. Hypoxia induces several transcription factors including HIF-1 (hypoxia inducible factor-1), which is composed of two sub-units HIF-1α and HIF-1β [196,200]. Under normal growth conditions HIF-1 is regulated by oxygen-dependent prolyl hydroxylases (PHDs) and the VHL ubiquitin ligase, which promote its proteosomal degradation [201]. However, HIF-1 becomes transcriptionally-active under low oxygen conditions. It was shown that under hypoxic conditions MnSOD suppresses the induction of HIF-1α in human breast carcinoma cells. This suggests that superoxide may contribute to HIF-1α accumulation [133]. However, increased generation of H_2O_2 also led to accumulation of HIF-1α, suggesting that both types of ROS can increase HIF-1α levels [133]. Increased HIF-1α expression has been shown to correlate with poor prognosis and increased cancer cell invasiveness. HIF-1 regulates glycolysis-related genes and inhibits mitochondrial respiration (reviewed in [196]), resulting in hypoxic adaption of tumour cells. This leads to glycolytic ATP generation [202], reduced formation of mitochondrially-generated H_2O_2, enhanced survival of poorly oxygenated cells and regulation of EMT- and metastasis-related genes [203]. HIF-1 also prevents intracellular acidification, which leads to an increased formation of lactate and CO_2 [202], both favouring extracellular matrix degradation and cell invasion [204].

Role of oxidative stress in angiogenesis

With increased tumour growth, more nascent blood vessels are developed to facilitate oxygen and nutrient supply to the centre of the tumour [205,206]. Several lines of evidence suggest a role for ROS in augmenting angiogenesis. For example, hypoxic conditions stimulate blood vessel development, whereby the blood flow in these new vessels is often chaotic, causing oxidative stress through periods of hypoxia and reoxygenation [181]. It was shown with a mouse model for breast cancer that administration of Mn(III) orthotetrakis-N-ethylpyridylporphyrin, a potent scavenger of reactive oxygen and nitrogen species, attenuates angiogenesis by modifying the density of microvessels and the proliferation rate of endothelial cells [207].

Angiogenesis is mediated through growth factors such as vesicular epithelial growth factor (VEGF) [208 210]. VEGF expression can be regulated by nutrient deprivation and hypoxia, which both increase intracellular levels of reactive oxygen species [211]. In such an environment HIF-1 and its co-factor p300 initiate gene expression including the expression of VEGF [212,213]. On the other hand, suppression of endogenous ROS by mitochondrial inhibitors or glutathione peroxidase decreases HIF-1 induction and VEGF expression in cancer cells [214]. Growth factor-mediated activation of Akt and subsequent formation of superoxide and H_2O_2 also lead to an induction of HIF-1 followed by expression of VEGF [86,215]. This is blocked when cells are pre-treated with catalase [86]. The knockdown of PTEN, a negative-regulatory phosphatase for the PI3K/Akt pathway, enhances VEGF secretion [216]. This is probably mediated by an increase in basal levels of hydrogen peroxide and superoxide, due to decreased expression of several antioxidant enzymes such as peroxiredoxins and Cu/ZnSOD [94].

ROS-induced secretion of matrix metalloproteinases such as MMP-1 from tumour cells promotes vessel growth within the tumour microenvironment. Further, a transient expression of MMP-1, MMP-2 and MMP-9 correlates with an increase in ROS during formation of capillary-like structures, implicating that MMP-mediated angiogenesis also occurs through upregulation of ROS [217]. ROS can also trigger vasodilation to increase the blood supply of tumours through activation of heme oxygenase-1, a enzyme that generates carbon monoxide or induces the formation of nitric oxide [218].

ROS and redox regulation in cancer stem cells

It is well established that after chemo- or radiotherapy a small sub-population of surviving primary cancer cells

can initiate recurrence. This sub-population of cells, termed cancer stem cells (CSC), expresses stem cell markers and is highly drug resistant. CSCs utilize redox-regulatory mechanisms to promote cell survival and tolerance to treatment [219,220]. As previously discussed, the accumulation of ROS is thought to contribute to the conversion of normal cells to cancer cells by mediating genomic instability, oncogenic growth, ECM independency and increased motility. In contrast to cancer cells, which maintain these high ROS levels during all stages of malignancy, cancer stem cells have an increased antioxidant capacity [221]. Keeping endogenous and induced ROS at moderate levels mediates drug resistance and allows these cells to survive during treatment, resulting in both stemness and cancer-initiating capabilities. Diehn et al. [222] recently showed that human and murine mammary epithelial cancer stem cells contain lower concentrations of ROS, specifically superoxide, than the more mature progeny, but also normal epithelial cells. They further demonstrated that these differences in ROS levels are critical for maintaining stem cell function. When compared to their normal tumour cell counterparts, CSCs showed increased expression of a variety of enzymes that contribute to oxygen radical scavenging [222]. Particularly genes regulating or involved in glutathione synthesis, including glutathione synthetases and glutamate cysteine ligase, were increased in their expression. Also increased was the expression of FOXO1, a forkhead transcription factor that was previously implicated in the regulation of other ROS scavengers such as SOD and catalase to confer resistance to oxidative stress in haematopoietic stem cells [223].

Since ROS are critical mediators of ionizing radiation-induced therapy [224,225] the expression of antioxidants in CSCs prevented DNA damage and protected cells from irradiation-induced cell death [222]. L-*S,R*-buthionine sulphoximine (BSO)-mediated pharmacological depletion of the ROS scavenger GSH in epithelial CSC markedly decreased their clonogenicity and resulted in increased radiosensitization [222]. Consequently, CSC-enriched populations accumulated fewer single and double strand breaks in their DNA after irradiation. Due to high levels of antioxidant signalling, cancer stem cells may also not be responsive to other (chemotherapeutic) treatments that target cancer cells by increasing intracellular ROS levels. To reduce recurrence in response to conventional therapy cancer stem cells have to be additionally targeted under consideration of their unique redox status. It will be interesting to see if decreasing oxidative defenses in cancer stem cells *in vivo* will cause them to loose their stemness, and if a combination therapy with standard chemotherapy is effective to eliminate both tumour and cancer stem cells.

Random damaging functions of ROS

Increased levels of reactive oxygen species can lead to 'non-specific' damage of macromolecules such as DNA, proteins and lipids. Some ROS such as H_2O_2 are not very reactive towards DNA and most of the damaging effects on DNA are due to hydroxyl ions, which are generated via the Fenton reaction [226]. In this reaction transition metals such as iron and copper donate or accept free electrons during intracellular reactions and use H_2O_2 to catalyse free radical formation. Hydroxyl radicals attack DNA rapidly due to their high diffusibility, which results in formation of DNA lesions including oxidized DNA bases, single strand and double strand breaks [227,228]. DNA adducts are removed by either the base excision repair (BER) or the nuclear excision repair (NER) pathways [229]. Cells incapable to completely repair DNA lesions (i.e. due to deficient DNA repair enzymes) undergo apoptosis to ensure these mutations will not be passed on to progeny cells. However, under certain circumstances, the cells harbouring DNA mutations successfully escape programmed cell death, which raises a high chance for cancerous growth.

The oxidative modification of proteins by reactive species is implicated in the aetiology or progression of various disorders and diseases. The major damage of ROS to proteins is modification in their amino acid residues, resulting in altered functions. Some ROS-induced modifications also increase protein carbonylation, nitration of tyrosine and phenylalanine residues, protein degradation [230] or lead to formation of cross-linked and glycated proteins [231,232]. The oxidized amino acid residues of proteins can influence their ability in signal transduction mechanisms. For example, irreversible oxidation of phosphatases within the catalytic sites hinders their enzymatic activity [233]. Oxidative alterations of enzymes also impact DNA repair efficiency, the fidelity of DNA polymerase during replication/synthesis and transcriptional activity, which tightly associates with cancer onset [1,234 236].

Other cellular targets of ROS are lipids. ROS react with polyunsaturated or polydesaturated fatty acids to initiate lipid peroxidation [237,238]. Lipid oxidation generates numerous genotoxic molecules such as malondialdehyde, 2-alkenals and 4-hydroxy-2-alkenals [239, 240]. ROS-induced lipid peroxidation can be used as a tumour marker, as shown in clinical studies [241]. For example, the detection of thiobarbituric acid-reactive substances in the serum of patients with colorectal cancer indicates a high level of lipid peroxidation.

Application of ROS and antioxidants in cancer therapy and prevention

Many chemotherapeutic strategies are designed to exuberantly-increase cellular ROS levels with the goal to induce irreparable damages, subsequently resulting in tumour cell apoptosis (for a detailed review on the use of ROS in cancer therapy see [221]). Dependent on the tumour type, this can be achieved through chemotherapy or radiation therapy [1,242 244]. For example,

for pancreatic cancer, to date only few treatment strategies have been proven as effective for therapy and these include combination therapy of gemcitabine with trichostatin A, epigallocate-3-gallate (EGCG), capsaicin and benzyl isothiocyanate (BITC) [148,245 249]. All of these drugs share the same mechanism, namely to elevate intracellular ROS levels to trigger apoptosis [146,148,250,251]. Another compound that modulates ROS levels and is currently tested for its potential use in tumour therapy is Sulindac, a FDA-approved, non-steroidal and anti-inflammatory drug. Sulindac enhances intracellular ROS levels and renders colon and lung cancer cells more sensitive to H_2O_2-induced apoptosis [252]. In addition, Aminoflavone (5-amino-2-(4-amino-3-fluorophenyl)-6,8-difluoro-7-methylchromen-4-one; AF) induces cell death in MCF-7 and MDA-MB-468 breast cancer cells, but is not toxic for non-malignant MCF-10A breast epithelial cells [253,254]. Upon treatment with Aminoflavone, an increase of intracellular ROS is detected, correlating with increased activation of Caspase 3 and subsequent apoptosis. The inhibition of ROS generation by pre-treatment of cells with N-acetyl-L-cysteine (NAC) reverses Aminoflavone-induced cell death [254]. Several compounds such as IOA, pancratistatin (PST) and triphala (TPL) induce apoptosis of breast cancer cells through similar mechanisms as Aminoflavone, which is to increase intracellular ROS levels through dissipation of the mitochondrial membrane potential [255 260].

Mitochondrial DNA codes for several respiratory chain sub-units and is more vulnerable to DNA damage than nuclear DNA. The exposure of cells to ionizing radiation can lead to mitochondrial complex II dysfunction and increase the steady state levels of reactive oxygen species and contribute to genomic instability [261]. In human cancer, mutations in mitochondrial genes, such as the gene encoding cytochrome *c* oxidase II, are associated with increased ROS generation [262]. However, the susceptibility of mitochondrial DNA to ROS-induced mutation may also be utilized for therapy. For example, chemotherapeutic treatment of cancer patients with DNA damaging agents can lead to cell death by inducing mutations in the mitochondrial DNA that increase cellular ROS to a toxic level [262].

As discussed above, when compared to normal cells, cancer cells show increased sensitivity to glucose-induced cytotoxicity and it was suggested that increased glucose metabolism in cancer cells can compensate excess metabolic production of ROS. For example, glucose metabolism inhibits apoptosis in cancer cells through redox inactivation of cytochrome *c* [263]. Therefore, it was concluded that inhibition of glucose metabolism may provide a target for selectively targeting cancer cells by enhancing their oxidative stress levels to promote cell death [264]. 2-deoxyglucose (2DG), a glucose analogue that can not be metabolized, increased oxidative stress levels and caused cell death in pancreatic and prostate cancer cells [265,266]. Moreover, this can be enhanced by additionally increasing cellular ROS levels with mitochondrial electron chain blockers [267].

Modulation of intracellular ROS levels can also be utilized to target oxidative stress-mediated tumour progression. For example, a loss of cell adhesion in tumour cells and anchorage-independent survival is tightly linked to a gain of cell motility and increased invasiveness. Salvicine (SAL) is a compound originally identified as a topoisomerase II poison and has been entered in a Phase II clinical trial for cancer therapy. Treatment of invasive MDA-MB-435 breast cancer cells with SAL causes rounded cell morphology, which indicates a decrease in cell adhesion [71]. The inhibition of ROS by the free radical scavenger NAC restores cell adhesion of MDA-MB-435 cells, suggesting that ROS augment their metastatic ability.

Since evidence from clinical and bench studies indicate that elevated intracellular ROS contribute to early events involved in cancer initiation and progression, an opposite approach to mediating an increase in cellular ROS levels is to use antioxidants to deplete tumour cells from ROS-induced survival signalling pathways. Such treatment may also have preventive functions. For instance, clinical studies have linked gain of oncogenic mutations in K-ras and subsequent ROS formation or pancreatic inflammation (pancreatitis) and macrophage-mediated generation of hydrogen peroxide and superoxide to events leading to an increased risk for pancreatic cancer [268 270]. Other examples are individuals with a high cancer risk due to the deficiency of inherited tumour suppressor genes such as p53 or PTEN. For these groups a treatment with antioxidants may be effective in delaying or even preventing tumour development. Depending on the therapeutic strategy, a use of antioxidants in combination therapy may have an adverse effect on anti-cancer drugs that act on tumor cells by increasing ROS levels to induce cell death. However, a combination therapy with antioxidants and therapeutics that induce apoptosis independent of oxidative stress may be effective. Antioxidants under development for clinical use are for example the SOD mimetic EUK-134 [271] or a mimetic of glutathione disulphide named NOV-002 [272].

In conclusion, to tailor specific combination therapy and to decide which strategy to use, chemotherapeutics that excessively increase intracellular ROS to reach a toxic level or antioxidants may be dependent on the tumour type and stage, the type and level of endogenous ROS as well as abundance of ROS-induced survival pathways.

Summary

After malignant transformation many cancer cells show a sustained increase in intrinsic generation of

reactive oxygen species, which maintains the oncogenic phenotype and drives tumour progression. Redox adaption through upregulation of anti-apoptotic and antioxidant molecules allows cancer cells to promote survival and to develop resistance to anti-cancer drugs. Little is known about how an increase in intracellular oxidative stress levels is sensed and transduced into ROS-induced specific intracellular signalling to regulate the expression of antioxidant and survival genes [142]. The dependence of tumour cells and cancer stem cells on their antioxidant capacity makes them vulnerable to agents that dampen antioxidant systems. There is a realistic prospect for treatments aimed to dramatically increase intracellular ROS to kill cancer cells by decreasing their antioxidant capacity [1]. This may be obtained using compounds that inhibit antioxidant systems or through inhibition of specific signalling pathways that upregulate antioxidants in cancer cells. The resulting increase in reactive oxygen species then may induce tumour cell death either through random damaging functions of ROS or by specific induction of apoptosis via death signalling pathways. The advantage of such a strategy is that normal cells are not significantly affected since they have lower basal ROS levels and therefore are less dependent on antioxidants. However, it is possible that a threshold of toxicity in these cancer cells is not reached and that the additional increase in ROS further causes more mutations or drives cell migration and invasion [221,273]. Therefore, a combination of inhibitors of antioxidant systems with pharmacological agents with pro-oxidant properties to increase ROS levels within tumour cells may be needed to overwhelm antioxidant systems over the threshold of toxicity [1,221]. It becomes evident that a much more detailed understanding of ROS-mediated signalling in tumour cells is necessary to develop new strategies for such a redox modulation-based therapeutic intervention to selectively kill cancer cells and overcome drug resistance.

Acknowledgements

The authors would like to thank Heike D ppler for critical reading of the manuscript.

***Declaration of interest*:** Research in the Storz laboratory is supported by grants from the Mayo Clinic SPORE for Pancreatic Cancer (P50 CA102701), the Mayo Clinic Breast Cancer SPORE (CA116201-03DR4), an AACR-PANCAN Career development grant (08-20-25-STOR), a R21 from the NCI (CA135102) as well as a Bankhead-Coley grant (FLA07BN-08) from the Florida Department of Health. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the review.

References

[1] Storz P. Reactive oxygen species in tumor progression. Front Biosci 2005;10:1881 1896.
[2] Szatrowski TP, Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res 1991;51:794 798.
[3] Babior BM. NADPH oxidase: an update. Blood 1999;93: 1464 1476.
[4] Han D, Williams E, Cadenas E. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. Biochem J 2001;353: 411 416.
[5] Crompton M. The mitochondrial permeability transition pore and its role in cell death. Biochem J 1999;341: 233 249.
[6] Storz P. Reactive oxygen species-mediated mitochondria-to-nucleus signaling: a key to aging and radical-caused diseases. Sci STKE 2006;2006:re3.
[7] Bienert GP, et al. Specific aquaporins facilitate the diffusion of hydrogen peroxide across membranes. J Biol Chem 2007; 282:1183 1192.
[8] Lee WK, Thevenod F. A role for mitochondrial aquaporins in cellular life-and-death decisions? Am J Physiol Cell Physiol 2006;291:C195-202.
[9] Dansen TB, Wirtz KW. The peroxisome in oxidative stress. IUBMB Life 2001;51:223 30.
[10] del Rio LA, et al. Metabolism of oxygen radicals in peroxisomes and cellular implications. Free Radic Biol Med 1992;13:557 580.
[11] Singh I. Mammalian peroxisomes: metabolism of oxygen and reactive oxygen species. Ann NY Acad Sci 1996;804:612 627.
[12] Bonekamp NA, et al. Reactive oxygen species and peroxisomes: struggling for balance. Biofactors 2009;35: 346 355.
[13] Bae YS, et al. Platelet-derived growth factor-induced H(2) O(2) production requires the activation of phosphatidylinositol 3-kinase. J Biol chem 2000;275:10527 10531.
[14] Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. Free Radic biol med 1995;18:775 794.
[15] Goustin AS, et al. Growth factors and cancer. Cancer Res 1986;46:1015 1029.
[16] Sundaresan M, et al. Requirement for generation of H2O2 for platelet-derived growth factor signal transduction. Science 1995;270:296 299.
[17] Tiku ML, Liesch JB, Robertson FM. Production of hydrogen peroxide by rabbit articular chondrocytes. Enhancement by cytokines. J Immunol 1990;145:690 696.
[18] Lo YY, Cruz TF. Involvement of reactive oxygen species in cytokine and growth factor induction of c-fos expression in chondrocytes. J Biol chem 1995;270:11727 11730.
[19] Meier B, et al. Human fibroblasts release reactive oxygen species in response to interleukin-1 or tumour necrosis factor-alpha. Biochem J 1989;263:539 545.
[20] Roy D, Sarkar S, Felty Q. Levels of IL-1 beta control stimulatory/inhibitory growth of cancer cells. Front Biosci 2006;11:889 898.
[21] Ohba M, et al. Production of hydrogen peroxide by transforming growth factor-beta 1 and its involvement in induction of egr-1 in mouse osteoblastic cells. J Cell Biol 1994;126:1079 1088.
[22] Chen Q, Olashaw N, Wu J. Participation of reactive oxygen species in the lysophosphatidic acid-stimulated mitogen-activated protein kinase kinase activation pathway. J biol chem 1995;270:28499 28502.
[23] Griendling KK, et al. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. Circ Res 1994;74:1141 1148.

[24] Aunoble B, et al. Major oncogenes and tumor suppressor genes involved in epithelial ovarian cancer (review). Int J Oncol 2000;16:567 576.

[25] Minamoto T, Mai M, Ronai Z. K-ras mutation: early detection in molecular diagnosis and risk assessment of colorectal, pancreas, and lung cancers a review. Cancer Detect Prev 2000;24:1 12.

[26] Minamoto T, Ougolkov AV, Mai M. Detection of oncogenes in the diagnosis of cancers with active oncogenic signaling. Expert Rev Mol Diagn 2002;2:565 575.

[27] Chiarugi P, Fiaschi T. Redox signalling in anchorage-dependent cell growth. Cell Signal 2007;19:672 682.

[28] Ferraro D, et al. Pro-metastatic signaling by c-Met through RAC-1 and reactive oxygen species (ROS). Oncogene 2006;25:3689 3698.

[29] Shin EA, et al. Arachidonic acid induces the activation of the stress-activated protein kinase, membrane ruffling and H2O2 production via a small GTPase Rac1. FEBS Lett 1999:452:355 359.

[30] Balkwill F. Tumour necrosis factor and cancer. Nat Rev Cancer 2009;9:361 371.

[31] Berasain C; et al. Inflammation and liver cancer: new molecular links. Ann NY Acad Sci 2009:1155:206 221.

[32] Xiao H, Yang CS. Combination regimen with statins and NSAIDs: a promising strategy for cancer chemoprevention. Int J Cancer 2008:123:983 990.

[33] Babior BM. The respiratory burst oxidase. Curr Opin Hematol 1995;2:55 60.

[34] Segal AW, Shatwell KP. The NADPH oxidase of phagocytic leukocytes. Ann NY Acad Sci1997;832:215 222.

[35] Cui S, et al. Activated murine macrophages induce apoptosis in tumor cells through nitric oxide-dependent or -independent mechanisms. Cancer Res 1994;54:2462 2467.

[36] Copin JC, Gasche Y, Chan PH. Overexpression of copper/zinc superoxide dismutase does not prevent neonatal lethality in mutant mice that lack manganese superoxide dismutase. Free Radic Biol Med 2000;28:1571 1576.

[37] Bendayan M, Reddy JK. Immunocytochemical localization of catalase and heat-labile enoyl-CoA hydratase in the livers of normal and peroxisome proliferator-treated rats. Lab Invest 1982;47:364 369.

[38] Hashimoto F, Hayashi H. Significance of catalase in peroxisomal fatty acyl-CoA beta-oxidation: NADH oxidation by acetoacetyl-CoA and H2O2. J Biochem 1990;108:426 431.

[39] Litwin JA, Volkl A, Muller-Hocker J, Hashimoto T, Fahimi H D. Immunocytochemical localization of peroxisomal enzymes in human liver biopsies. Am J Pathol 1987;128: 141 150.

[40] Hofmann B, Hecht HJ, Flohe L. Peroxiredoxins. Biol Chem 2002;383:347 364.

[41] Rhee SG, et al. A family of novel peroxidases, peroxiredoxins. Biofactors 1999;10:207 209.

[42] Wood ZA, et al. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem Sci 2003;28:32 40.

[43] Neumann CA, et al. Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. Nature 2003;424:561 565.

[44] Holmgren A. Thioredoxin structure and mechanism: conformational changes on oxidation of the active-site sulfhydryls to a disulfide. Structure 1995;3:239–243.

[45] Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000;267:6102 6109.

[46] Mustacich D, Powis G. Thioredoxin reductase. Biochemical J 2000;346:1 8.

[47] Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. Free Radic biol Med 1999;27:951 965.

[48] Ursini F, et al. Diversity of glutathione peroxidases. Methods in Enzymol 1995;252:38 53.

[49] Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J Biol Chem 1975;250:5475 5480.

[50] Beutler E. Effect of flavin compounds on glutathione reductase activity: in vivo and in vitro studies. J Clin Invest 1969;48:1957 1966.

[51] Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene 2003;22:7369 7375.

[52] Sharma R, et al. Antioxidant role of glutathione S-transferases: protection against oxidant toxicity and regulation of stress-mediated apoptosis. Antioxid & Redox signal 2004;6:289 300.

[53] Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005;45:51 88.

[54] Colavitti R, et al. Reactive oxygen species as downstream mediators of angiogenic signaling by vascular endothelial growth factor receptor-2/KDR. J Biol Chem 2002;277: 3101 3108.

[55] Finkel T. Redox-dependent signal transduction. FEBS Lett 2000;476:52 54.

[56] Hensley K, Floyd RA. Reactive oxygen species and protein oxidation in aging: a look back, a look ahead. Arch Biochem Biophys 2002;397:377 383.

[57] Irani K. Oxidant signaling in vascular cell growth, death, and survival : a review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. Circulation Res 2000; 87:179 183.

[58] Rhee SG, et al. Hydrogen peroxide: a key messenger that modulates protein phosphorylation through cysteine oxidation. Sci STKE 2000:53:pel.

[59] Khavari TA, Rinn J. Ras/Erk MAPK signaling in epidermal homeostasis and neoplasia. Cell Cycle 2007;6:2928 2931.

[60] Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007;26:3291 3310.

[61] Irani K, et al. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science 1997;275:1649–1652.

[62] Reddy KB, Glaros S. Inhibition of the MAP kinase activity suppresses estrogen-induced breast tumor growth both in vitro and in vivo. Int J Oncol 2007;30:971 975.

[63] Lander HM, et al. A molecular redox switch on p21(ras). Structural basis for the nitric oxide-p21(ras) interaction. J Biol chem 1997;272:4323 4326.

[64] Chan DW, et al. Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. Carcinogenesis 2008;29:1742 1750.

[65] McCubrey JA, et al . Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 2007;1773:1263 1284.

[66] Steelman LS, et al. Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia 2008;22:686 707.

[67] Kumar B, et al. Oxidative stress is inherent in prostate cancer cells and is required for aggressive phenotype. Cancer Res 2008;68:1777 1785.

[68] Lee WC, et al. Role of ERK in hydrogen peroxide-induced cell death of human glioma cells. Neurochem Res 2005; 30:263 270.

[69] Rygiel TP, et al. The Rac activator Tiam1 prevents keratinocyte apoptosis by controlling ROS-mediated ERK phosphorylation. J Cell Sci 2008;121:1183 1192.

[70] Ostrakhovitch EA, Cherian MG. Inhibition of extracellular signal regulated kinase (ERK) leads to apoptosis inducing factor (AIF) mediated apoptosis in epithelial breast cancer cells: the lack of effect of ERK in p53 mediated copper induced apoptosis. J cell biochem 2005;95:1120 1134.

[71] Zhou J, et al. Salvicine inactivates beta 1 integrin and inhibits adhesion of MDA-MB-435 cells to fibronectin via reactive oxygen species signaling. Mol Cancer Res 2008; 6:194 204.
[72] Cullen JJ, et al. The role of manganese superoxide dismutase in the growth of pancreatic adenocarcinoma. Cancer Res 2003;63:1297 1303.
[73] Lewis A, et al. Metastatic progression of pancreatic cancer: changes in antioxidant enzymes and cell growth. Clin Exp Metastasis 2005;22:523 532.
[74] Mazzio EA, Soliman KF. Glioma cell antioxidant capacity relative to reactive oxygen species produced by dopamine. J Appl Toxicol 2004;24:99 106.
[75] Zhang Y, et al. Overexpression of copper zinc superoxide dismutase suppresses human glioma cell growth. Cancer Res 2002;62:1205 1212.
[76] Osada S, et al. Extracellular signal-regulated kinase phosphorylation due to menadione-induced arylation mediates growth inhibition of pancreas cancer cells. Cancer Chemother Pharmacol 2008;62:315 320.
[77] Shi Y, Sahu RP, Srivastava SK. Triphala inhibits both in vitro and in vivo xenograft growth of pancreatic tumor cells by inducing apoptosis. BMC Cancer 2008;8:294.
[78] Brunet A, et al. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell 1999;96:857 868.
[79] Pastorino JG, Tafani M, Farber JL. Tumor necrosis factor induces phosphorylation and translocation of BAD through a phosphatidylinositide-3-OH kinase-dependent pathway. J Biol Chem 1999;274:19411 19416.
[80] Xin M, Deng X. Nicotine inactivation of the proapoptotic function of Bax through phosphorylation. J Biol Chem 2005;280:10781 10789.
[81] Kawamura N, et al. Akt1 in osteoblasts and osteoclasts controls bone remodeling. PLoS One 2007;2:e1058.
[82] Qi XJ, Wildey GM, Howe PH. Evidence that Ser87 of BimEL is phosphorylated by Akt and regulates BimEL apoptotic function. J Biol Chem 2006;281:813 823.
[83] Limaye V, et al. Sphingosine kinase-1 enhances endothelial cell survival through a PECAM-1-dependent activation of PI-3K/Akt and regulation of Bcl-2 family members. Blood 2005;105:3169 3177.
[84] Burdick AD, et al. Benzo(a)pyrene quinones increase cell proliferation, generate reactive oxygen species, and transactivate the epidermal growth factor receptor in breast epithelial cells. Cancer Res 2003;63:7825 7833.
[85] Park SA, et al. 4-hydroxyestradiol induces anchorage-independent growth of human mammary epithelial cells via activation of IkappaB kinase: potential role of reactive oxygen species. Cancer Res 2009;69:2416 2424.
[86] Liu LZ, et al. Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1alpha expression through activation of AKT and P70S6K1 in human ovarian cancer cells. Free Radic Biol Med 2006;41:1521 1533.
[87] Mochizuki T, et al. Inhibition of NADPH oxidase 4 activates apoptosis via the AKT/apoptosis signal-regulating kinase 1 pathway in pancreatic cancer PANC-1 cells. Oncogene 2006;25:3699 3707.
[88] Storz P, Toker A. 3 -phosphoinositide-dependent kinase-1 (PDK-1) in PI 3-kinase signaling. Front Biosci 2002;7: d886 d902.
[89] Ogg S, Ruvkun G. The C. elegans PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol Cell 1998;2:887 893.
[90] Sun H, et al. PTEN modulates cell cycle progression and cell survival by regulating phosphatidylinositol 3,4,5,-trisphosphate and Akt/protein kinase B signaling pathway. Proc Natl Acad Sci USA 1999;96:6199 6204.
[91] Higaki Y, et al. Oxidative stress stimulates skeletal muscle glucose uptake through a phosphatidylinositol 3-kinase-dependent pathway. Am J Physiol Endocrinol Metab 2008;294:E889 E897.
[92] Prasad N, et al. Oxidative stress and vanadate induce tyrosine phosphorylation of phosphoinositide-dependent kinase 1 (PDK1). Biochemistry 2000;39:6929 6935.
[93] Lee SR, et al. Reversible inactivation of the tumor suppressor PTEN by H2O2. J Biol Chem 2002;277:20336 20342.
[94] Huo YY, et al. PTEN deletion leads to deregulation of antioxidants and increased oxidative damage in mouse embryonic fibroblasts. Free Radic Biol Med 2008;44: 1578 1591.
[95] Basseres DS, Baldwin AS. Nuclear factor-kappaB and inhibitor of kappaB kinase pathways in oncogenic initiation and progression. Oncogene 2006;25:6817 6830.
[96] Biswas DK, Iglehart JD. Linkage between EGFR family receptors and nuclear factor kappaB (NF-kappaB) signaling in breast cancer. J Cell Physiol 2006;209:645 652.
[97] Biswas DK, et al. NF-kappa B activation in human breast cancer specimens and its role in cell proliferation and apoptosis. Proc Natl Acad Sci USA 2004;101:10137 10142.
[98] Rayet B, Gelinas C. Aberrant rel/nfkb genes and activity in human cancer. Oncogene 1999;18:6938 6947.
[99] Ahmed KM, Cao N, Li JJ. HER-2 and NF-kappaB as the targets for therapy-resistant breast cancer. Anticancer Res 2006;26:4235 4243.
[100] Bourguignon LY, Xia W, Wong G. Hyaluronan-mediated CD44 interaction with p300 and SIRT1 regulates beta-catenin signaling and NFkappaB-specific transcription activity leading to MDR1 and Bcl-xL gene expression and chemoresistance in breast tumor cells. J Biol Chem 2009;284:2657 2671.
[101] Duffey DC, et al. Inhibition of transcription factor nuclear factor-kappaB by a mutant inhibitor-kappaBalpha attenuates resistance of human head and neck squamous cell carcinoma to TNF-alpha caspase-mediated cell death. Br J Cancer 2000;83:1367 1374.
[102] Li N, Karin M. Is NF-kappaB the sensor of oxidative stress? Faseb J 1999;13:1137 1143.
[103] Schreck R, Albermann K, Baeuerle PA. Nuclear factor kappa B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic Res Commun 1992;17:221 237.
[104] Beg AA, et al. Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of I kappa B alpha: a mechanism for NF-kappa B activation. Mol Cell Biol 1993;13: 3301 3310.
[105] Beg AA, et al. I kappa B interacts with the nuclear localization sequences of the subunits of NF-kappa B: a mechanism for cytoplasmic retention. Genes Dev 1992;6:1899 1913.
[106] Bonizzi G, et al. Activation of IKKalpha target genes depends on recognition of specific kappaB binding sites by RelB:p52 dimers. Embo J 2004;23:4202 4210.
[107] Delhase M, et al. Positive and negative regulation of IkappaB kinase activity through IKKbeta subunit phosphorylation. Science 1999;284:309 313.
[108] Hacker H, Karin M. Regulation and function of IKK and IKK-related kinases. Sci STKE 2006;357:re13.
[109] Bergmann M, et al. IkappaBalpha degradation and nuclear factor-kappaB DNA binding are insufficient for interleukin-1beta and tumor necrosis factor-alpha-induced kappaB-dependent transcription. Requirement for an additional activation pathway. J Biol Chem 1998;273: 6607 6610.
[110] Claudio E, et al. Molecular mechanisms of TNFalpha cytotoxicity: activation of NF-kappaB and nuclear translocation. Exp Cell Res 1996;224:63 71.

[111] Ling L, Cao Z, Goeddel DV. NF-kappaB-inducing kinase activates IKK-alpha by phosphorylation of Ser-176. Proc Natl Acad Sci USA 1998;95:3792 3797.
[112] Ghoda L, Lin X, Greene WC. The 90-kDa ribosomal S6 kinase (pp90rsk) phosphorylates the N-terminal regulatory domain of IkappaBalpha and stimulates its degradation in vitro. J Biol Chem 1997;272:21281 21288.
[113] Singh S, Darnay BG, Aggarwal BB. Site-specific tyrosine phosphorylation of IkappaBalpha negatively regulates its inducible phosphorylation and degradation. J Biol Chem 1996;271:31049 31054.
[114] Karin M. The IkappaB kinase-a bridge between inflammation and cancer. Cell Res 2008;18:334 342.
[115] Janssen-Heininger YM, Poynter ME, Baeuerle PA. Recent advances towards understanding redox mechanisms in the activation of nuclear factor kappaB. Free Radic Biol Med 2000;28:1317 1327.
[116] Li Q, Engelhardt JF. Interleukin-1beta induction of NFkappaB is partially regulated by H_2O_2-mediated activation of NFkappa B-inducing kinase. J Biol Chem 2006;281:1495 1505.
[117] Manna SK, et al. Overexpression of manganese superoxide dismutase suppresses tumor necrosis factor-induced apoptosis and activation of nuclear transcription factor-kappaB and activated protein-1. J Biol Chem 1998;273:13245 13254.
[118] Ruiz-Ramos R, et al. Sodium arsenite induces ROS generation, DNA oxidative damage, HO-1 and c-Myc proteins, NF-kappaB activation and cell proliferation in human breast cancer MCF-7 cells. Mutat Res 2009;674:109 115.
[119] Wang Y, et al. The endogenous reactive oxygen species promote NF-kappaB activation by targeting on activation of NF-kappaB-inducing kinase in oral squamous carcinoma cells. Free Radic Res 2007;41:963 971.
[120] Storz P, Doppler H, Toker A. Protein kinase Cdelta selectively regulates protein kinase D-dependent activation of NF-kappaB in oxidative stress signaling. Mol Cell Biol 2004;24:2614 2626.
[121] Storz P, Toker A. Protein kinase D mediates a stress-induced NF-kappaB activation and survival pathway. The EMBO J 2003;22:109 120.
[122] Cowell CF, et al. Mitochondrial diacylglycerol initiates protein-kinase D1-mediated ROS signaling. J Cell Sci 2009;122:919 928.
[123] Doppler H, Storz P. A novel tyrosine phosphorylation site in protein kinase D contributes to oxidative stress-mediated activation. J Biol chem 2007;282:31873 31881.
[124] Storz P, Doppler H, Toker A. Activation loop phosphorylation controls protein kinase D-dependent activation of nuclear factor kappaB. Mol Pharmacol 2004;66:870 879.
[125] Imbert V, et al. Tyrosine phosphorylation of I kappa B-alpha activates NF-kappa B without proteolytic degradation of I kappa B-alpha. Cell 1996;86:787 798.
[126] Mukhopadhyay A, Manna SK, Aggarwal BB. Pervanadate-induced nuclear factor-kappaB activation requires tyrosine phosphorylation and degradation of IkappaBalpha. Comparison with tumor necrosis factor-alpha. J Biol chem 2000;275:8549 8555.
[127] Gupta A, Rosenberger SF, Bowden GT. Increased ROS levels contribute to elevated transcription factor and MAP kinase activities in malignantly progressed mouse keratinocyte cell lines. Carcinogenesis 1999;20:2063 2073.
[128] Burdon RH, Gill V, Rice-Evans C. Oxidative stress and tumour cell proliferation. Free Radic Res Commun 1990; 11:65 76.
[129] Felty Q, Roy D. Estrogen, mitochondria, and growth of cancer and non-cancer cells. J Carcinog 2005;4:1.
[130] Felty Q, Singh KP, Roy D. Estrogen-induced G1/S transition of G0-arrested estrogen-dependent breast cancer cells is regulated by mitochondrial oxidant signaling. Oncogene 2005;24:4883 4893.
[131] Felty Q, et al. Estrogen-induced mitochondrial reactive oxygen species as signal-transducing messengers. Biochemistry 2005;44:6900 6909.
[132] Sarsour EH, et al. Manganese superoxide dismutase activity regulates transitions between quiescent and proliferative growth. Aging cell 2008;7:405 417.
[133] Wang M, et al. Manganese superoxide dismutase suppresses hypoxic induction of hypoxia-inducible factor-1alpha and vascular endothelial growth factor. Oncogene 2005;24: 8154 8166.
[134] Parkash J, Felty Q, Roy D. Estrogen exerts a spatial and temporal influence on reactive oxygen species generation that precedes calcium uptake in high-capacity mitochondria: implications for rapid nongenomic signaling of cell growth. Biochemistry 2006;45:2872 2881.
[135] Menon SG, et al. Differential susceptibility of nonmalignant human breast epithelial cells and breast cancer cells to thiol antioxidant-induced G(1)-delay. Antioxid Redox Signal 2005;7:711 718.
[136] Behrend L, Henderson G, Zwacka RM. Reactive oxygen species in oncogenic transformation. Biochem Soc Trans 2003;31:1441 1444.
[137] Browne SE, et al. Treatment with a catalytic antioxidant corrects the neurobehavioral defect in ataxia-telangiectasia mice. Free Radic Biol Med 2004;36:938 942.
[138] Reichenbach J, et al. Elevated oxidative stress in patients with ataxia telangiectasia. Antioxidants & redox signaling 2002;4:465 469.
[139] Cadenas E. Mitochondrial free radical production and cell signaling. Mol Aspects Med 2004;25:17 26.
[140] Simon HU, Haj-Yehia A, Levi-Schaffer F. Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 2000;5:415 418.
[141] Chung YM, Bae YS, Lee SY. Molecular ordering of ROS production, mitochondrial changes, and caspase activation during sodium salicylate-induced apoptosis. Free Radic Biol Med 2003;34:434 442.
[142] Storz P. Mitochondrial ROS–radical detoxification, mediated by protein kinase D. Trends Cell Biol 2007;17:13 18.
[143] Gottlieb E, Vander Heiden MG, Thompson C. B. Bcl-x(L) prevents the initial decrease in mitochondrial membrane potential and subsequent reactive oxygen species production during tumor necrosis factor alpha-induced apoptosis. Mol Cell Biol 2000;20:5680 5689.
[144] Li PF, Dietz R, von Harsdorf R. p53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome c-independent apoptosis blocked by Bcl-2. EMBO J 1999;18:6027 6036.
[145] Lee CH, et al. Novel 2-step synthetic indole compound 1,1,3-tri(3-indolyl)cyclohexane inhibits cancer cell growth in lung cancer cells and xenograft models. Cancer 2008;113: 815 825.
[146] Qanungo S, et al. Epigallocatechin-3-gallate induces mitochondrial membrane depolarization and caspase-dependent apoptosis in pancreatic cancer cells. Carcinogenesis 2005;26:958 967.
[147] Shim H, et al. Acacetin-induced apoptosis of human breast cancer MCF-7 cells involves caspase cascade, mitochondria-mediated death signaling and SAPK/JNK1/2-c-Jun activation. Mol Cells 2007;24:95 104.
[148] Zhang R, et al. In vitro and in vivo induction of apoptosis by capsaicin in pancreatic cancer cells is mediated through ROS generation and mitochondrial death pathway. Apoptosis 2008;13:1465 1478.
[149] Saitoh M, et al. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. EMBO J 1998;17:2596 2606.
[150] Takeda K, et al. Roles of MAPKKK ASK1 in stress-induced cell death. Cell Struct Funct 2003;28:23 29.

[151] You H, Yamamoto K, Mak TW. Regulation of transactivation-independent proapoptotic activity of p53 by FOXO3a. Proc Natl Acad Sci USA 2006;103:9051 9056.
[152] Schulze-Osthoff K, et al. Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO J 1993;12:3095 3104.
[153] Xu YC, et al. Involvement of TRAF4 in oxidative activation of c-Jun N-terminal kinase. J Biol Chem 2002;277: 28051 28057.
[154] Wong GH, Goeddel DV. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. Science 1988;242:941 944.
[155] Chiu TT, et al. Protein kinase D2 mediates lysophosphatidic acid-induced interleukin 8 production in nontransformed human colonic epithelial cells through NF-kappaB. Am J Physiol Cell Physiol 2007;292:C767 C777.
[156] Mihailovic T, et al. Protein kinase D2 mediates activation of nuclear factor kappaB by Bcr-Abl in Bcr-Abl+ human myeloid leukemia cells. Cancer Res 2004;64:8939 8944.
[157] Song J, et al. PKD prevents H2O2-induced apoptosis via NF-kappaB and p38 MAPK in RIE-1 cells. Biochem Biophys Res Commun 2009;378:610 614.
[158] Storz P, et al. Functional dichotomy of A20 in apoptotic and necrotic cell death. Biochem J 2005;387:47 55.
[159] Storz P, Doppler H, Toker A. Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species. Mol Cell Biol 2005;25:8520 8530.
[160] Zhang W, et al. Protein kinase D specifically mediates apoptosis signal-regulating kinase 1-JNK signaling induced by H2O2 but not tumor necrosis factor. J Biol Chem 2005;280: 19036 19044.
[161] Kundu N, et al. Sublethal oxidative stress inhibits tumor cell adhesion and enhances experimental metastasis of murine mammary carcinoma. Clin Exp Metastasis 1995;13:16 22.
[162] Pelicano H, et al. Mitochondrial dysfunction and reactive oxygen species imbalance promote breast cancer cell motility through a CXCL14-mediated mechanism. Cancer Res 2009;69:2375 2383.
[163] Hitchler M. J, et al. Epigenetic silencing of SOD2 by histone modifications in human breast cancer cells. Free Radic Biol Med 2008;45:1573 1580.
[164] Hitchler MJ, et al. Epigenetic regulation of manganese superoxide dismutase expression in human breast cancer cells. Epigenetics 2006;1:163 171.
[165] Broom OJ, Massoumi R, Sjolander A. Alpha2beta1 integrin signalling enhances cyclooxygenase-2 expression in intestinal epithelial cells. J Cell Physiol 2006;209:950 958.
[166] Svineng G, et al. The role of reactive oxygen species in integrin and matrix metalloproteinase expression and function. Connect Tissue Res 2008;49:197 202.
[167] Taddei ML, et al. Integrin-mediated cell adhesion and spreading engage different sources of reactive oxygen species. Antioxid Redox Signal 2007;9:469 481.
[168] Ben Mahdi MH, Andrieu V, Pasquier C. Focal adhesion kinase regulation by oxidative stress in different cell types. IUBMB Life 2000;50:291 299.
[169] Chiarugi P. From anchorage dependent proliferation to survival: lessons from redox signalling. IUBMB Life 2008;60:301 307.
[170] Werner E, Werb Z. Integrins engage mitochondrial function for signal transduction by a mechanism dependent on Rho GTPases. J Cell Biol 2002;158:357 368.
[171] Giannoni E, et al. Redox regulation of anoikis resistance of metastatic prostate cancer cells: key role for Src and EGFR-mediated pro-survival signals. Oncogene 2009;28: 2074 2086.
[172] Radisky DC, et al. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 2005;436:123 127.
[173] Wu WS. The signaling mechanism of ROS in tumor progression. Cancer Metastasis Rev 2006;25:695 705.
[174] Tobar N, et al. RAC1 activity and intracellular ROS modulate the migratory potential of MCF-7 cells through a NADPH oxidase and NFkappaB-dependent mechanism. Cancer Lett 2008;267:125 132.
[175] Moldovan L, et al. The actin cytoskeleton reorganization induced by Rac1 requires the production of superoxide. Antioxid Redox signal 1999;1:29 43.
[176] Eiseler T, et al. Protein kinase D1 regulates cofilin-mediated F-actin reorganization and cell motility through slingshot. Nat cell Biol 2009;11:545 556.
[177] Kim JS, Huang TY, Bokoch GM. Reactive oxygen species regulate a slingshot-cofilin activation pathway. Mol Biol Cell 2009;20:2650 2660.
[178] Sundaresan M, et al. Regulation of reactive-oxygen-species generation in fibroblasts by Rac1. Biochem J 1996;318: 379 382.
[179] Gianni D, et al. The involvement of the tyrosine kinase c-Src in the regulation of reactive oxygen species generation mediated by NADPH oxidase-1. Mol Biol Cell 2008;19:2984 2994.
[180] Diaz B, et al. Tks5-dependent, nox-mediated generation of reactive oxygen species is necessary for invadopodia formation. Sci Signal 2009;2:ra53.
[181] Brown NS, Bicknell R. Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Res 2001;3:323 327.
[182] Duffy MJ, et al. Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. Breast Cancer Res 2000;2:252 257.
[183] Nelson AR, et al. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000;18:1135 1149.
[184] Nelson KK, Melendez J. A. Mitochondrial redox control of matrix metalloproteinases. Free Radic Biol Med 2004;37: 768 784.
[185] Brenneisen P, Sies H, Scharffetter-Kochanek K. Ultraviolet-B irradiation and matrix metalloproteinases: from induction via signaling to initial events. Ann NY Acad Sci 2002;973: 31 43.
[186] Kheradmand F, et al. Role of Rac1 and oxygen radicals in collagenase-1 expression induced by cell shape change. Science 1998;280:898 902.
[187] Wenk J, et al. Stable overexpression of manganese superoxide dismutase in mitochondria identifies hydrogen peroxide as a major oxidant in the AP-1-mediated induction of matrix-degrading metalloprotease-1. J Biol Chem 1999;274: 25869 25876.
[188] Westermarck J, Kahari VM. Regulation of matrix metalloproteinase expression in tumor invasion. Faseb J 1999;13:781 792.
[189] Rajagopalan S, et al. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 1996;98: 2572 2579.
[190] van Wetering S, et al. Reactive oxygen species mediate Rac-induced loss of cell-cell adhesion in primary human endothelial cells. J Cell Sci 2002;115:1837 1846.
[191] Cheng GC, et al. Oxidative stress and thioredoxin-interacting protein promote intravasation of melanoma cells. Exp Cell Res 2004;300:297 307.
[192] Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB (Review). Int J Mol Med 1999;4:223 230.
[193] Huot J, et al. Oxidative stress-induced actin reorganization mediated by the p38 mitogen-activated protein kinase/heat shock protein 27 pathway in vascular endothelial cells. Circ Res 1997;80:383 392.

[194] Dewhirst MW, Cao Y, Moeller B. Cycling hypoxia and free radicals regulate angiogenesis and radiotherapy response. Nat Rev Cancer 2008;8:425 437.
[195] Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001;93:266 276.
[196] Pani G, et al. Redox-based escape mechanism from death: the cancer lesson. Antioxidants Redox Signal 2009;11: 2791 2806.
[197] Dang CV, Semenza GL. Oncogenic alterations of metabolism. Trends Biochem Sci 1999;24:68 72.
[198] Ganapathy V, Thangaraju M, Prasad P. D. Nutrient transporters in cancer: relevance to Warburg hypothesis and beyond. Pharmacol Ther 2009;121:29 40.
[199] Hsu PP, Sabatini DM. Cancer cell metabolism: Warburg and beyond. Cell 2008;134:703 707.
[200] Harris AL. Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38 47.
[201] Kaelin WG, Jr. Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. Mol Cell 2008;30:393 402.
[202] Pouyssegur J, Dayan F, Mazure NM. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 2006;441:437 443.
[203] Erler JT, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. Nature 2006;440:1222 1226.
[204] Rofstad EK, et al. Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. Cancer Res 2006;66:6699 6707.
[205] Claffey KP, et al. Expression of vascular permeability factor/ vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. Cancer Res 1996;56:172 181.
[206] Senger DR, et al. Vascular permeability factor, tumor angiogenesis and stroma generation. Invasion Metastasis 1994;14:385 394.
[207] Rabbani ZN, et al. Antiangiogenic action of redox-modulating Mn(III) ortho-tetrakis-N-ethylpyridylporphyrin, MnTE-2-PyP(5+), via suppression of oxidative stress in a mouse model of breast tumor. Free Radic Biol Med 2009.
[208] Brown LF, et al. Vascular permeability factor/vascular endothelial growth factor: a multifunctional angiogenic cytokine. Exs 1997;79:233 269.
[209] Murakami M, Simons M. Fibroblast growth factor regulation of neovascularization. Curr Opin Hematol 2008;15:215 220.
[210] Wouters BG, Koritzinsky M. Hypoxia signalling through mTOR and the unfolded protein response in cancer. Nat Rev Cancer 2008;8:851 864.
[211] Spitz DR, et al. Glucose deprivation-induced oxidative stress in human tumor cells. A fundamental defect in metabolism? Ann NY Acad Sci 2000;899:349 362.
[212] Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer. Nat Rev Cancer 2008;8:967 975.
[213] Gardner LB, Corn, PG. Hypoxic regulation of mRNA expression. Cell Cycle 2008;7:1916 1924.
[214] Xia C, et al. Reactive oxygen species regulate angiogenesis and tumor growth through vascular endothelial growth factor. Cancer Res 2007;67:10823 10830.
[215] Govindarajan B, et al. Overexpression of Akt converts radial growth melanoma to vertical growth melanoma. J Clin Invest 2007;117:719 729.
[216] Ma J, et al. PTEN regulate angiogenesis through PI3K/Akt/ VEGF signaling pathway in human pancreatic cancer cells. Mol Cell Biochem; 2009.
[217] Wartenberg M, et al. Inhibition of tumor-induced angiogenesis and matrix-metalloproteinase expression in confrontation cultures of embryoid bodies and tumor spheroids by plant ingredients used in traditional chinese medicine. Lab Invest 2003;83:87 98.
[218] Milligan SA, Owens MW, Grisham MB. Augmentation of cytokine-induced nitric oxide synthesis by hydrogen peroxide. Am J Physiol 1996;271:L114 L120.
[219] Bao S, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 2006;444:756 760.
[220] Visvader JE, Lindeman GJ. Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. Nat Rev Cancer 2008;8:755 768.
[221] Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov 2009;8:579 591.
[222] Diehn M, et al. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 2009;458:780 783.
[223] Tothova Z, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell 2007;128:325 339.
[224] Shackleton M, et al. Generation of a functional mammary gland from a single stem cell. Nature 2006;439:84 88.
[225] Ward JF. Biochemistry of DNA lesions. Radiat Res Suppl 1985;8:S103 S111.
[226] Imlay JA, Chin SM, Linn S. Toxic DNA damage by hydrogen peroxide through the Fenton reaction in vivo and in vitro. Science 1988;240:640 642.
[227] Maynard S, et al. Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis 2009;30:2 10.
[228] Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 1996;313:17 29.
[229] Mitra S, et al. Complexities of the DNA base excision repair pathway for repair of oxidative DNA damage. Environ Mol Mutagen 2001;38:180 190.
[230] Levine RL. Carbonyl modified proteins in cellular regulation, aging, and disease. Free Radic Biol Med 2002;32:790 796.
[231] Squier TC, Bigelow DJ. Protein oxidation and age-dependent alterations in calcium homeostasis. Front Biosci 2000:5:D504 D526.
[232] Wells-Knecht MC, et al. Age-dependent increase in ortho-tyrosine and methionine sulfoxide in human skin collagen is not accelerated in diabetes. Evidence against a generalized increase in oxidative stress in diabetes. J Clin Invest 1997;100:839 846.
[233] Meng TC, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatases in vivo. Molecular Cell 2002;9:387 399.
[234] Burhans WC, Weinberger M. DNA replication stress, genome instability and aging. Nucleic Acids Res 2007;35:7545 7556.
[235] Epe B. Role of endogenous oxidative DNA damage in carcinogenesis: what can we learn from repair-deficient mice? Biol Chem 2002;383:467 475.
[236] Franco R, et al. Oxidative stress, DNA methylation and carcinogenesis. Cancer Lett 2008;266:6 11.
[237] Gardner HW. Oxygen radical chemistry of polyunsaturated fatty acids. Free Radic Biol Med 1989;7:65 86.
[238] North JA, Spector AA, Buettner GR. Cell fatty acid composition affects free radical formation during lipid peroxidation. Am J Physiol 1994;267:C177 C188.
[239] Burcham PC. Genotoxic lipid peroxidation products: their DNA damaging properties and role in formation of endogenous DNA adducts. Mutagenesis 1998;13:287 305.
[240] Catala A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem Phys Lipids 2009;157:1 11.
[241] Lauschke H, et al. Lipid peroxidation as additional marker in patients with colorectal cancer. Results of a preliminary study. Eur Surg Res 2002;34:346 350.

[242] Alexandre J, et al. Accumulation of hydrogen peroxide is an early and crucial step for paclitaxel-induced cancer cell death both in vitro and in vivo. Int J Cancer 2006;119:41 48.
[243] Bairati I, et al. Randomized trial of antioxidant vitamins to prevent acute adverse effects of radiation therapy in head and neck cancer patients. J Clin Oncol 2005;23:5805 5813.
[244] Llobet D, et al. Antioxidants block proteasome inhibitor function in endometrial carcinoma cells. Anticancer Drugs 2008;19:115 124.
[245] Gahr S, et al. The combination of the histone-deacetylase inhibitor trichostatin A and gemcitabine induces inhibition of proliferation and increased apoptosis in pancreatic carcinoma cells. Int J Oncol 2007;31:567 576.
[246] Lee KH, et al. Epigenetic silencing of MicroRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer. Pancreatology 2009;9:293 301.
[247] Shankar S, et al. EGCG inhibits growth, invasion, angiogenesis and metastasis of pancreatic cancer. Front Biosci 2008;13:440 452.
[248] Shankar S, Suthakar G, Srivastava RK. Epigallocatechin-3-gallate inhibits cell cycle and induces apoptosis in pancreatic cancer. Front Biosci 2007;12:5039 5051.
[249] Srivastava SK, Singh SV. Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. Carcinogenesis 2004;25: 1701 1709.
[250] Donadelli M, et al. Synergistic inhibition of pancreatic adenocarcinoma cell growth by trichostatin A and gemcitabine. Biochim Biophys Acta 2007;1773:1095 1106.
[251] Sahu RP, et al. Benzyl isothiocyanate mediated generation of reactive oxygen species causes cell cycle arrest and induces apoptosis via activation of MAPK in human pancreatic cancer cells. Carcinogenesis; 2009.
[252] Marchetti M, et al. Sulindac enhances the killing of cancer cells exposed to oxidative stress. PLoS One 2009;4:e5804.
[253] Loaiza-Perez AI, et al. Aryl hydrocarbon receptor activation of an antitumor aminoflavone: basis of selective toxicity for MCF-7 breast tumor cells. Mol Cancer Ther 2004;3: 715 725.
[254] McLean L, et al. Aminoflavone induces oxidative DNA damage and reactive oxidative species-mediated apoptosis in breast cancer cells. Int J Cancer 2008;122:1665 1674.
[255] Chen CY, et al. Isokotomolide A, a new butanolide extracted from the leaves of Cinnamomum kotoense, arrests cell cycle progression and induces apoptosis through the induction of p53/p21 and the initiation of mitochondrial system in human non-small cell lung cancer A549 cells. Eur J Pharmacol 2007;574:94 102.
[256] Kuo PL, Chen CY, Hsu YL. Isoobtusilactone A induces cell cycle arrest and apoptosis through reactive oxygen species/apoptosis signal-regulating kinase 1 signaling pathway in human breast cancer cells. Cancer Res 2007;67: 7406 7420.
[257] McLachlan A, et al. Pancratistatin: a natural anti-cancer compound that targets mitochondria specifically in cancer cells to induce apoptosis. Apoptosis 2005;10:619 630.
[258] Sandhya T, et al. Potential of traditional ayurvedic formulation, Triphala, as a novel anticancer drug. Cancer Lett 2006;231:206 214.
[259] Sandhya T, Mishra KP. Cytotoxic response of breast cancer cell lines, MCF 7 and T 47 D to triphala and its modification by antioxidants. Cancer Lett 2006;238:304 313.
[260] Siedlakowski P, et al. Synergy of Pancratistatin and Tamoxifen on breast cancer cells in inducing apoptosis by targeting mitochondria. Cancer Biol Ther 2008;7:376 384.
[261] Dayal D, et al. Mitochondrial complex II dysfunction can contribute significantly to genomic instability after exposure to ionizing radiation. Radiat Res 2009;172:737 745.
[262] Carew JS, et al. Mitochondrial DNA mutations in primary leukemia cells after chemotherapy: clinical significance and therapeutic implications. Leukemia 2003;17:1437 1447.
[263] Vaughn AE, Deshmukh M. Glucose metabolism inhibits apoptosis in neurons and cancer cells by redox inactivation of cytochrome c. Nat Cell Biol 2008;10:1477 1483.
[264] Aykin-Burns N, et al. Increased levels of superoxide and H2O2 mediate the differential susceptibility of cancer cells versus normal cells to glucose deprivation. Biochem J 2009;418:29 37.
[265] Ahmad IM, et al. 2-Deoxyglucose combined with wild-type p53 overexpression enhances cytotoxicity in human prostate cancer cells via oxidative stress. Free Radic Biol Med 2008;44:826 834.
[266] Coleman MC, et al. 2-deoxy-D-glucose causes cytotoxicity, oxidative stress, and radiosensitization in pancreatic cancer. Free Radic Biol Med 2008;44:322 331.
[267] Fath MA, et al. Mitochondrial electron transport chain blockers enhance 2-deoxy-D-glucose induced oxidative stress and cell killing in human colon carcinoma cells. Cancer Biol Ther 2009;8:1228 1236.
[268] Algul H, et al. Mechanisms of disease: chronic inflammation and cancer in the pancreas--a potential role for pancreatic stellate cells? Nat Clin Pract Gastroenterol Hepatol 2007;4:454 462.
[269] Greer JB,Whitcomb DC. Inflammation and pancreatic cancer: an evidence-based review. Curr Opin Pharmacol; 2009.
[270] Lowenfels AB, et al. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. N Engl J Med 1993;328:1433 1437.
[271] Antosiewicz J, et al. c-Jun NH(2)-terminal kinase signaling axis regulates diallyl trisulfide-induced generation of reactive oxygen species and cell cycle arrest in human prostate cancer cells. Cancer Res 2006;66:5379 5386.
[272] Townsend DM, Tew KD. Pharmacology of a mimetic of glutathione disulfide, NOV-002. Biomed Pharmacother 2009; 63:75 78.
[273] Storz P, et al. FOXO3a promotes tumor cell invasion through the induction of matrix metalloproteinases. Mol Cell Biol 2009;29:4906 4917.

This paper was first published online on Early Online on 23 March 2010.

Exhibit 65

Published in final edited form as:
Cell. 2010 March 19; 140(6): 883–899. doi:10.1016/j.cell.2010.01.025.

Immunity, Inflammation, and Cancer

Sergei I. Grivennikov[1], **Florian R. Greten**[2], and **Michael Karin**[1]

[1]Laboratory of Gene Regulation and Signal Transduction, Departments of Pharmacology and Pathology, School of Medicine, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093, USA.

[2]2nd Department of Medicine, Klinikum rechts der Isar, Technical University Munich, Munich, Germany.

Summary

Inflammatory responses play decisive roles at different stages of tumor development, including initiation, promotion, malignant conversion, invasion, and metastasis. Inflammation also affects immune surveillance and responses to therapy. Immune cells that infiltrate tumors engage in an extensive and dynamic crosstalk with cancer cells and some of the molecular events that mediate this dialog have been revealed. This review outlines the principal mechanisms that govern the effects of inflammation and immunity on tumor development and discusses attractive new targets for cancer therapy and prevention.

Keywords

Cancer; inflammation; immunity; cytokines; NF-κB; STAT3

Introduction

The presence of leukocytes within tumors, observed in the 19th century by Rudolf Virchow, provided the first indication of a possible link between between inflammation and cancer. Yet, it is only during the last decade that clear evidence has been obtained that inflammation plays a critical role in tumorigenesis, and some of the underlying molecular mechanisms have been elucidated (Karin, 2006). A role for inflammation in tumorigenesis is now generally accepted, and it has become evident that an inflammatory microenvironment is an essential component of all tumors, including some in which a direct causal relationship with inflammation is not yet proven (Mantovani et al., 2008). Only a minority of all cancers are caused by germline mutations, whereas the vast majority (90%) are linked to somatic mutations and environmental factors. Many environmental causes of cancer and risk factors are associated with some form of chronic inflammation. Up to 20% of cancers are linked to chronic infections, 30% can be attributed to tobacco smoking and inhaled pollutants (such as silica and asbestos), and 35% to dietary factors (20% of cancer burden is linked to obesity) (Aggarwal et al., 2009).

Corresponding author: Michael Karin karinoffice@ucsd.edu .

Authors declare no competing financial interests.

Although it is now well-established that the induction of inflammation by bacterial and viral infections increases cancer risk (de Martel and Franceschi, 2009), recent work has shown that in addition to being a tumor initiator by virtue of its high carcinogen content, tobacco smoke is also a tumor promoter due to its ability to trigger chronic inflammation (Takahashi et al., 2010). Likewise, obesity, whose prevalence is growing at an alarming rate, promotes tumorigenesis in the liver (Park et al., 2010) and pancreas (Khasawneh et al., 2009). Most solid malignancies appear in older individuals and even old age (Ershler and Keller, 2000) and cell senescence (Rodier et al., 2009) are postulated to be tumor promoters that act through inflammatory mechanisms. Along with its pro-tumorigenic effects, inflammation also influences the host immune response to tumors and can be used in cancer immunotherapy (Dougan and Dranoff, 2009) and to augment the response to chemotherapy (Zitvogel et al., 2008). Yet, in some cases, inflammation can diminish the beneficial effects of therapy (Ammirante et al., 2010). This review is mainly focused on the pro-tumorigenic effects of inflammation but also touches on the relationship between inflammation and anti-tumor immunity,

Types of inflammation and general mechanisms

Several types of inflammation—differing by cause, mechanism, outcome, and intensity—can promote cancer development and progression (Figure 1). Persistent *Helicobacter pylori* infection is associated with gastric cancer and MALT (mucosa-associated lymphoid tissue) lymphoma. Infections with hepatitis B (HBV) or C (HCV) viruses increase the risk of hepatocellular carcinoma (HCC) and infections with *Schistosoma* or *Bacteroides* species are linked to bladder and colon cancer, respectively (Karin, 2006;Wu et al., 2009a). The inflammatory response triggered by infection precedes tumor development and is a part of normal host defense, whose goal is pathogen elimination. However, tumorigenic pathogens subvert host immunity and establish persistent infections associated with low grade but chronic inflammation. By contrast, acute inflammation induced by certain microbial preparations was used by Coley with some success to treat cancer in the 1890s and one such preparation is currently used in the treatment of bladder cancer (Rakoff-Nahoum and Medzhitov, 2009). What makes bladder carcinoma uniquely sensitive to acute inflammation, even though it is promoted by chronic inflammation, is currently unknown. This is an important problem whose solution should reveal how to successfully deploy inflammation in cancer therapy. Another type of chronic inflammation that precedes tumor development is caused by immune deregulation and autoimmunity. An example is inflammatory bowel disease, which greatly increases the risk of colorectal cancer (Waldner and Neurath, 2009).

However, not all chronic inflammatory diseases increase cancer risk and some of them, such as psoriasis, may even reduce it (Nickoloff et al., 2005). It is not clear what makes IBD or chronic hepatitis tumor promoting, in comparison with conditions such as rheumatoid arthritis or psoriasis, which do not significantly promote tumorigenesis. One possibility could be related to the exposure of the gastrointestinal tract and liver to dietary and environmental carcinogens, which never make their way into joints or the skin. Chronic inflammation can also be induced by environmental exposure. Particulate material from tobacco smoke and other irritants can precipitate chronic obstructive pulmonary disease, a condition associated with higher lung cancer risk (Punturieri et al., 2009). Inflammatory mechanisms account for the tumor promoting effect of exposure to tobacco smoke on lung cancer in mice (Takahashi et al., 2010). Inhaled asbestos or silica particles also give rise to lung cancer but have no obvious mutagenic activity. Such particles, however, can trigger inflammation through effects on pro-interluekin-1□(IL-1□) processing by the inflammasome (Dostert et al., 2008) and this may mediate their tumorigenic activity. Even obesity, which increases cancer risk by 1.6-fold (Calle, 2007), can lead to chronic inflammation (Tuncman et al., 2006) that promotes development of hepatocellular carcinoma (Park et al., 2010). Accumulation of damaged DNA and cell

senescence can also give rise to tumor promoting chronic inflammation (Rodier et al., 2009; Zheng et al., 2007).

A completely different type of inflammation is the one that follows tumor development. Most, if not all, solid malignancies trigger an intrinsic inflammatory response that builds up a pro-tumorigenic microenvironment (Mantovani et al., 2008). In addition to cell-autonomous proliferation, certain oncogenes, such as *RAS* and *MYC* family members, induce a transcriptional program that leads to remodeling of the tumor microenvironment through recruitment of leukocytes and lymphocytes, expression of tumor-promoting chemokines and cytokines, and induction of an angiogenic switch (Soucek et al., 2007; Sparmann and Bar-Sagi, 2004). All solid malignancies, at some point outpace their blood supply and become oxygen and nutrient deprived. This results in necrotic cell death at the tumor's core and the release of pro-inflammatory mediators, such as IL-1 and HMGB1 (Vakkila and Lotze, 2004). The ensuing inflammatory response promotes neo-angiogenesis and provides surviving cancer cells with additional growth factors, produced by newly recruited inflammatory and immune cells (Karin, 2006).

Other tumors, for instance lung cancer, can promote inflammation through active secretion of molecules, such as the extracellular matrix component versican that activates macrophages through Toll-like receptor (TLR) 2 (Kim et al., 2009). Based on the continuous cell renewal and proliferation induced by tumor-associated inflammation, tumors have been referred to as "wounds, which never heal" (Dvorak, 1986). This type of inflammation is largely a subverted wound healing and tissue regenerative response. Even dominant oncogenes such as v-Src or K-Ras are unable to induce cancer in adult animals unless accompanied by injury and subsequent tissue regeneration (Guerra et al., 2007; Sieweke et al., 1990).

Lastly, a strong tumor-associated inflammatory response can be initiated by cancer therapy. Radiation and chemotherapy cause massive necrotic death of cancer cells and surrounding tissues, which in turn trigger an inflammatory reaction analogous to a wound-healing response (Zong and Thompson, 2006). The net outcome of therapy-induced inflammation is controversial, as on one hand it can have tumor-promoting functions just like the necrosis that accompanies rapid tumor growth (Ammirante et al., 2010; Vakkila and Lotze, 2004), but on the other hand it can enhance the cross-presentation of tumor antigens and subsequent induction of an anti-tumor immune response (Zitvogel et al., 2008). The latter and its importance will be discussed below.

Immune cells in tumorigenesis

As a result of these different forms of inflammation, the tumor microenvironment contains innate immune cells (including macrophages, neutrophils, mast cells, myeloid derived suppressor cells, dendritic cells, and natural killer cells) and adaptive immune cells (T and B lymphocytes) in addition to the cancer cells and their surrounding stroma (which consists of fibroblasts, endothelial cells, pericytes, and mesenchymal cells) (de Visser et al., 2006) (Table 1). These diverse cells communicate with each other by means of direct contact or cytokine and chemokine production and act in autocrine and paracrine manners to control and shape tumor growth. It is the expression of various immune mediators and modulators as well as the abundance and activation state of different cell types in the tumor microenvironment that dictate in which direction the balance is tipped and whether inflammation-promotes tumor growth or anti-tumor immunity will ensue (Lin and Karin, 2007; Smyth et al., 2006). In established tumors this balance is profoundly tilted towards pro-tumor inflammation, as without therapeutic intervention advanced tumors rarely regress. Yet, it is difficult to unequivocally assess the overall impact of immunity and inflammation on early tumorigenic events, because direct in vivo models for evaluating the effects of these phenomena on initial

tumor growth are missing. In addition, our current knowledge is based on measurement of tumor load at a point where malignant cells may have already escaped early surveillance mechanisms. However, it is safe to assume that tumor promoting inflammation and anti-tumor immunity co-exist at different points along the path of tumor progression (Figure 2) and that environmental and microenvironmental conditions dictate the balance between the two (Bui and Schreiber, 2007; Swann et al., 2008).

The most frequently found immune cells within the tumor microenvironment are tumor-associated macrophages (TAMs) and T cells. TAMs mostly promote tumor growth and may be obligatory for angiogenesis, invasion, and metastasis (Condeelis and Pollard, 2006), and high TAM content generally correlates with poor prognosis (Murdoch et al., 2008). Mature T cells are divided into two major groups based on the T cell receptors (TCR) they express: □□ and □□. □□T cells are further classified according to their effector functions as $CD8^+$ cytotoxic T cells (CTLs) and $CD4^+$ helper T (Th) cells, which include Th1, Th2, Th17 and T regulatory (Treg) cells, as well as natural killer T (NKT) cells. Importantly, T cells can exert both tumor suppressive and promoting effects, as determined by their effector functions (DeNardo et al., 2009; Langowski et al., 2007; Smyth et al., 2006). Increased T cell numbers, specifically activated CTLs and Th cells, correlate with better survival in some cancers, including invasive colon cancer, melanoma, multiple myeloma, and pancreatic cancer (Galon et al., 2006; Laghi et al., 2009; Swann and Smyth, 2007). Correspondingly, T cell deficiency or disruption of specific cytotoxic mechanisms can render experimental animals more susceptible to spontaneous or chemical carcinogenesis (Shankaran et al., 2001; Swann and Smyth, 2007). However, there is also evidence that many of the T cell subsets found in solid tumors are involved in tumor promotion, progression, or metastasis, including $CD8^+$ T cells (Roberts et al., 2007), IFN□-producing Th1 cells (Hanada et al., 2006), Th2 cells (Aspord et al., 2007; DeNardo et al., 2009) and Th17 cells (Langowski et al., 2006; Wang et al., 2009). The only cells that lack a pro-tumorigenic role, so far, are NK cells. Similar to TAMs, the tumor-promoting functions of T lymphocytes are mediated by cytokines, whereas both cytokines and cytotoxic mechanisms mediate the anti-tumorigenic functions of T lymphocytes (Lin and Karin, 2007; Swann and Smyth, 2007).

Interestingly, Treg cells, which are presumed to act mostly in a pro-tumorigenic fashion through suppression of anti-tumor immune responses (Gallimore and Simon, 2008), may also exert an anti-tumorigenic function under certain circumstances by virtue of their ability to suppress tumor-promoting inflammation (Erdman et al., 2005). In breast cancer, the presence of tumor infiltrating lymphocytes with high $CD4^+/CD8^+$ and Th2/Th1 ratio is indicative of poor prognosis (Kohrt et al., 2005). Th2 $CD4^+$ T cells stimulate mammary cancer progression and metastasis by educating TAMs to produce pro-angiogenic and pro-metastatic factors (DeNardo et al., 2009). In colitis associated cancer (CAC), infiltrating T cells also appear to play a tumor promoting function (Waldner and Neurath, 2009). What makes the same T cell subset anti-tumorigenic in one cancer and pro-tumorigenic in another remains largely unknown and may hold the key to the development of successful immunotherapy.

The cytokine and chemokine expression profile of the tumor microenvironment may be more relevant than its specific immune cell content. Different cytokines can either promote or inhibit tumor development and progression, regardless of their source (Lin and Karin, 2007). Through activation of various downstream effectors, such as NF-□B, AP-1, STAT and SMAD transcription factors, as well as caspases, cytokines control the immune and inflammatory milieu to either favor anti-tumor immunity (IL-12, TRAIL, IFN□) or enhance tumor progression (IL-6, IL-17, IL-23) and also have direct effects on cancer cell growth and survival (TRAIL, FasL, TNF-□, EGFR ligands, TGF-□, IL-6).

TAMs are one of the most important players in the inflammation and cancer arena and an important source of cytokines (Mantovani et al., 2008). In analogy to Th1 and Th2 T cells, macrophages can be classified into M1 and M2 types (Sica et al., 2008). M1 macrophages, activated by IFN□and microbial products, express high levels of pro-inflammatory cytokines (TNF-□, IL-1, IL-6, IL-12 or IL-23), major histocompatibility complex (MHC) molecules and inducible nitric oxide synthase and are capable of killing pathogens and priming anti-tumor immune responses. By contrast, M2 or "alternatively" activated macrophages, which are induced in vitro by IL-4, IL-10 and IL-13, downregulate MHC class II and IL-12 expression and show increased expression of the anti-inflammatory cytokine IL-10, scavenger receptor A, and arginase. Most TAMs are considered to have an M2 phenotype while promoting tumor angiogenesis and tissue remodeling (Sica et al., 2008). However, most confirmed tumor-promoting cytokines are "M1 cytokines", whereas IL-10, an M2 cytokine, may be tumor suppressive as shown in in colorectal cancer (Berg et al., 1996; Lin and Karin, 2007). Furthermore, unlike Th1 and Th2 cells, M1 and M2 macrophages are plastic and their phenotype is defined by their gene expression prolife rather than by deterministic differentiation pathways and lineage choices.

Other immune cells also affect tumorigenesis (Table 1). Neutrophils can play both tumor-promoting and tumoricidal functions, depending on their differentiation status and the presence of TGF-□(Fridlender et al., 2009). B lymphocytes and mast cells are also important contributors to immune-mediated tumor growth (Ammirante et al., 2010;de Visser et al., 2006;Soucek et al., 2007) and conventional macrophages and dendritic cells are important for antigen presentation and T cell activation during anti-tumor immunity as well as for cytokine production and immunosuppression in established tumors (Table 1).

Inflammation and tumor initiation

Tumor initiation is a process in which normal cells acquire the first mutational hit that sends them on the tumorigenic track by providing growth and survival advantages over their neighbors. In most cases, however, a single mutation is insufficient and many cancers require at least 4-5 mutations (Fearon and Vogelstein, 1990; Hanahan and Weinberg, 2000). It is also imperative that each mutation will be transmitted to the cell's progeny, and in cancers that arise within rapidly renewed epithelia (intestinal and skin cancers), oncogenic mutations must occur in either long lived stem cells or transient amplifying cells rather than within differentiated cells, which are rapidly eliminated before the next mutation can strike. Alternatively, oncogenic mutations can occur within differentiated epithelial cells, such as hepatocytes, which are capable of proliferation and are sufficiently long lived to allow subsequent mutational hits.

It has been suggested that an inflammatory microenvironment can increase mutation rates, in addition to enhancing the proliferation of mutated cells. Activated inflammatory cells serve as sources of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) that are capable of inducing DNA damage and genomic instability (Figure 3A). However, it is not clear whether ROS and RNI produced and released by neutrophils or macrophages (mainly during acute inflammation) are sufficiently long lived to diffuse through the extracellular matrix, enter epithelial cells, cross their cytoplasm, enter the nucleus and react with DNA packaged into chromatin. Alternatively, inflammatory cells may use cytokines such as TNF-□to stimulate ROS accumulation in neighboring epithelial cells (Figure 3A). It has therefore been debated whether immune-mediated mechanisms as opposed to dietary and environmental mutagens are the critical driving forces behind tumor initiation (Hussain et al., 2003). Nonetheless, p53 mutations, presumably caused by oxidative damage, were found in both cancer cells and in inflamed, but non-dysplastic, epithelium in CAC, suggesting that chronic inflammation causes genomic changes (Kraus and Arber, 2009). Chronic inflammation triggered by the colonic irritant dextran sodium sulfate (DSS) may induce DNA damage that gives rise to colonic

adenomas (Meira et al., 2008). However, on its own DSS is a poor carcinogen (Okayasu et al., 1996).

Inflammation-induced mutagenesis may also result in inactivation or repression of mismatch repair response genes and ROS can also cause direct oxidative inactivation of mismatch repair enzymes (Colotta et al., 2009; Hussain et al., 2003). Once the mismatch repair system has been dismantled, inflammation-induced mutagenesis is enhanced and several important tumor suppressors, such as Tgfbr2 and Bax, which harbor microsatellite sequences, may be inactivated (Colotta et al., 2009).

Another mechanism linking inflammation to oncogenic mutations is upregulation of AID (activation-induced cytidine deaminase), an enzyme that promotes immunoglobulin gene class switching by catalyzing deamination of cytosines in DNA (Okazaki et al., 2007). In addition to B cells, where it was discovered, AID is overexpressed in many cancers of diverse origins and its expression is induced by inflammatory cytokines in an NF-□B-dependent manner or by TGF□(Okazaki et al., 2007). AID induces genomic instability and increases mutation probability during error-prone joining of double-stranded DNA breaks, a process found to introduce mutations into critical cancer genes, including Tp53, c-Myc, and Bcl-6 (Colotta et al., 2009). AID contributes to formation of lymphomas, and gastric and liver cancers (Okazaki et al., 2007; Takai et al., 2009). Other mechanisms of inflammation-induced mutagenesis have also been suggested, including effects of inflammation on non-homologous recombination and NF-□B-mediated inactivation of p53-dependent genome surveillance (Colotta et al., 2009).

In *Gi□2* knockout mice, which develop spontaneous colonic inflammation and cancer, enterocytes selectively lose expression of components involved in mismatch repair, namely MLH1 and PMS2, due to histone deacetylase- and DEC-1-mediated epigenetic repression of the *Mlh1* promoter (Edwards et al., 2009). Other findings implicate epigenetic mechanisms, including microRNA-based silencing and DNA methylation, in inactivation of tumor suppressors, such as INK4a and APC, and other changes that accompany tumor initiation (Cooper and Foster, 2009). Recently, inflammation has been connected to epigenetic reprogramming by the JmjC-domain protein Jmjd3, which is encoded by an NF-□B target gene (De Santa et al., 2007). In inflammation-associated intestinal cancer in *Gpx1/2* knockout mice, inflammation induces DNA methyltransferase (DNMT)-dependent DNA methylation and silencing of a large cohort of Polycomb group target genes, some of which are also silenced by methylation in human colon cancer (Hahn et al., 2008). However, it remains to be shown that any of these inflammation-induced epigenetic mechanisms actually makes a critical contribution to tumor initiation, either in a suitable mouse model or through prospective analysis of human specimens.

Another mechanism through which inflammation may enhance tumor initiation is the production of growth factors and cytokines that can confer a stem-cell like phenotype upon tumor progenitors or stimulate stem cell expansion, thereby enlarging the cell pool that is targeted by environmental mutagens. Indeed, STAT3 is linked to both stem cell reprogramming and stem cell renewal (Chen et al., 2008), whereas NF-□B can enhance Wnt/□-catenin signaling in colonic crypts (Umar et al., 2009). The pro-inflammatory cytokine TNF-□promotes nuclear entry of □-catenin during inflammation-associated gastric cancer in the absence of any mutations in Wnt/□-catenin pathway components (Oguma et al., 2008).

The connection between inflammation and tumor initiation is not a one-way street and there is also evidence that DNA damage can lead to inflammation and thereby promote tumorigenesis. One of the best examples is provided by the model of hepatocellular carcinoma induced by the carcinogen diethylnitrosamine (DEN) in which DNA damage contributes to necrotic cell death, resulting in an inflammatory reaction that promotes tumor development

(Maeda et al., 2005; Sakurai et al., 2008). A number of oncoproteins (Ras, Myc, RET) can activate signaling pathways that drive production of pro-inflammatory cytokines and chemokines (IL-6, IL-8, IL-1□, CCL2, CCL20) (Mantovani et al., 2008). Genotoxic stress can also induce expression of NKG2D family members, which serve as ligands for NK and □□T cell receptors (Strid et al., 2008) resulting in either elimination of stressed cells or a local inflammatory response. In the same vein, mosaic deletion of the DNA repair gene ATR and Tp53 in the skin results in recruitment of $CD11b^{+}Gr1^{+}$ myeloid cells, as a part of a prototypical immune response to "altered self" (Ruzankina et al., 2009). Defective DNA repair caused by a deficiency of the Fen1 exonuclease also results in a tumor promoting inflammatory response that is driven by damaged DNA, most likely through activation of a pattern recognition receptor (Zheng et al., 2007).

Inflammation and tumor promotion

Tumor promotion is the process of tumor growth from a single initiated cell into a fully developed primary tumor. Initial tumor growth depends on increased cell proliferation and reduced cell death, both of which are stimulated by inflammation-driven mechanisms. In fact, many of the enhancing effects of inflammation on cancer are exerted at the level of tumor promotion and most known tumor promoters, for instance phorbol esters, are potent inducers of inflammation (Karin, 2006). Inflammation-induced tumor promotion may occur early or late in tumor development and can lead to activation of pre-malignant lesions that were dormant for many years. The mechanisms through which inflammation affects tumor promotion are numerous and in addition to increased proliferation and enhanced survival, can also involve the so-called angiogenic switch, which allows a small dormant tumor to receive the blood supply necessary for the next growth phase (Lewis and Pollard, 2006). Mechanisms of inflammation-driven tumor promotion are discussed below.

Tumor promoting cytokine signaling

Production of tumor promoting cytokines by immune/inflammatory cells that activate transcription factors, such as NF-□B, STAT3 and AP-1, in pre-malignant cells to induce genes that stimulate cell proliferation and survival, is a major tumor promoting mechanism (Figure 3B). Initial evidence for inflammation-mediated tumor promotion came from mouse models of skin, colon, and liver cancer. Although counterintuitive at the time, TNF-□was found to be required for two-stage skin carcinogenesis (Moore et al., 1999). TNF-□activates both AP-1 and NF-□B transcription factors, but in the skin its tumor promoting effects are mediated by AP-1 (Eferl and Wagner, 2003), which was identified as a transcription factor whose activity is stimulated by the classic tumor promoter tetradecanoyl phorbol acetate (TPA) (Angel et al., 1987). By contrast, NF-□B inhibits the development of skin cancer (Zhang et al., 2004). Thus, although a given cytokine may activate several transcription factors, its tumor promoting activity may be mediated by only one of them and antagonized by another. As discussed below, a similar situation may apply to liver cancer. Amongst the different transcription factors that are part of this mechanism, NF-□B and STAT3 are activated in the majority of cancers and act as non-classical oncogenes, whose activation in malignant cells is rarely the result of direct mutations, and instead depends on signals produced by neighboring cells or more rarely on mutational activation of upstream signaling components. NF-□B and STAT3 activate genes that control cell survival, proliferation, and growth, as well as angiogenesis, invasiveness, motility, chemokine, and cytokine production (Grivennikov and Karin, 2009; Yu et al., 2009).

Oncogenic transcription factors can also be activated through pattern recognition receptors by components of bacteria and viruses (Rakoff-Nahoum and Medzhitov, 2009). However, the overall contribution of pattern recognition receptors on epithelial cells versus those expressed

by immune/inflammatory cells to tumor promotion is far from being clear and will require the analysis of cell type specific knockout mice. Even the specific agonists that activate these receptors in cancer are not defined. Nonetheless, the role of the cytokines that are produced in response to damage-associated (DAMP) or pathogen-associated (PAMP) molecular patterns in tumor development is more firmly established. For example, AP-1 activation in skin cancer is largely dependent on TNF-TNFR1 signaling (Balkwill, 2009), whereas STAT3 activation in cancer cells is largely dependent on a plethora of growth factors and cytokines including IL-6, IL-11, IL-22, HGF, and EGF, and oncogenic tyrosine kinases, such as c-Met and Src (Bollrath et al., 2009; Grivennikov et al., 2009; Naugler et al., 2007; Yu et al., 2009).

The first critical genetic evidence for inflammatory cells as a source of tumor promoting cytokines was obtained in a mouse model of CAC, where inactivation of NF-□B in myeloid cells reduced tumor growth and blocked production of IL-6 and other cytokines in response to colitis (Greten et al., 2004). Subsequent work demonstrated that the effect of immune cells (macrophages, T cells) on CAC growth is mediated through IL-6, IL-11, TNF-□and IL-1□ (Becker et al., 2004; Bollrath et al., 2009; Grivennikov et al., 2009; Popivanova et al., 2008), as well as other cytokines, such as IL-23. IL-11 plays a similar role in gastric cancer (Ernst et al., 2008), in which IL-1□is also a tumor promoter (Tu et al., 2008). TNF-□also promotes HCC in mice lacking the P-glycoprotein Mdr2, which develop cholestatic inflammation followed by hepatocellular carcinoma (HCC) (Pikarsky et al., 2004). HCC can also be promoted by another member of the TNF family, lymphotoxin □(Haybaeck et al., 2009). TNF-□along with IL-6 contributes to obesity-mediated tumor promotion in HCC (Park et al., 2010). The latter effect correlates with the ability of TNF-□and IL-6 to promote hepatosteatosis and steatohepatitis (Park et al., 2010). One of the most critical tumor promoting cytokines in HCC is IL-6. Mice deficient in IL-6 develop much less HCC in response to the chemical pro-carcinogen DEN and the gender-biased production of IL-6 accounts for the much higher HCC load in males (Naugler et al., 2007). High levels of circulating IL-6, are associated with HCC risk factors, including hepatosteatosis, obesity, and liver cirrhosis, and are the best predictors of rapid progression from viral hepatitis to HCC in humans (Wong et al., 2009).

In CAC and HCC, the tumor promoting effect of IL-6 is mainly exerted via STAT3, whose cell type specific inactivation in hepatocytes and enterocytes inhibits the development of these malignancies in mice treated with DEN or azoxymethane (AOM) and DSS, respectively (Bollrath et al., 2009; Grivennikov et al., 2009; Park et al., 2010). Development of CAC in mice is also dependent on IKK□-mediated NF-□B activation in enterocytes, whose major function in this model is increased survival of pre-malignant cells (Greten et al., 2004). A similar role was proposed for NF-□B in HCC development in mice deficient in *Mdr2* and in lymphotoxin-transgenic mice both of which exhibit chronic liver inflammation (Haybaeck et al., 2009). However, in the DEN model of HCC and *Helicobacter*-driven gastric cancer, NF-□B promotes hepatocyte and epithelial cell survival and acts as an inhibitor of tumor development (Maeda et al., 2005; Shibata et al., 2009). Most likely, the diverse effects of NF-□B in different models are determined by the mechanism of tumor induction and the type of inflammatory response involved in tumor promotion. *Mdr2* knockout and lymphotoxin-transgenic mice exhibit a very low level of normal hepatocyte death, which is not enhanced by the absence of NF-□B (Haybaeck et al., 2009; Pikarsky et al., 2004). In these mice, NF-□B in hepatocytes is mainly responsible for propagating inflammation through induction of chemokines, which recruit immune/inflammatory cells into the liver. By contrast, DEN treated mice exhibit an acute inflammatory response triggered by IL-1□release from necrotic hepatocytes (Sakurai et al., 2008). IL-1□induces IL-6 production by Kupffer cells and this response drives the compensatory proliferation of surviving hepatocytes (a type of a wound-healing response); the greater the amount of cell death – the greater the regenerative response. By suppressing accumulation of ROS and preventing hepatocyte necrosis, NF-□B inhibits HCC induction in DEN treated mice (Maeda et al., 2005).

Another tumor-promoting cytokine is IL-23 (Langowski et al., 2006). IL-23 is mostly expressed by TAMs in a manner dependent on STAT3 and NF-□B (Kortylewski et al., 2009). IL-23 blockade with neutralizing antibodies or genetic inactivation of the IL-23p19 gene dramatically decrease tumor multiplicity and growth in the two-step model of skin carcinogenesis (Langowski et al., 2006). In part, the pro-tumorigenic effects of IL-23 may be mediated by IL-17 and IL-22 production by Th17 cells, but other effects of IL-23 on CTLs, Tregs, and myeloid cells should not be discounted. A close relative of IL-23 is IL-12, which shares with IL-23 the IL-12p40 subunit and is involved in Th1 differentiation, IFN□production, and activation of anti-tumor immunity (Trinchieri et al., 2003). Secretion of IL-23 and IL-12 secretion are reciprocally regulated and the switch from IL-12 to IL-23 production may be an important tumor promoting event. STAT3 activation, PGE_2, ATP, and lactic acid increase IL-23 production by TAMs (Kortylewski et al., 2009; Shime et al., 2008). The latter two agonists link cancer cell necrosis (induced by hypoxia or therapy) and the Warburg effect (the switch from oxidative phosphorylation to glycolysis) to IL-23 production, thereby shifting anti-tumor immunity to tumor promotion.

A similar circuit can be executed by myeloid-derived suppressor cells (MDSC) that produce arginase1 and indoleamine-2,3-dioxygenase, which are enzymes that dampen anti-tumor immunity through interference with T cell activation (Gabrilovich and Nagaraj, 2009). Taken together, tumor associated inflammation drives tumor growth, angiogenesis and can perpetuate itself through an extensive network of cytokines and chemokines, which are produced by immune, stromal and malignant cells in response to diverse signals (Figure 3B).

Given that several cytokines (IL-1, TNF, IL-6, IL-23) and transcription factors (AP-1, NF-□B, STAT3) are critical for both inflammation and tumor growth, they control hubs of pro-tumorigenic signaling that may be targeted to curtail both tumor associated inflammation and tumor growth (see below). Pharmacological interference with cytokine signaling decreases tumorigenesis as well as cancer growth (Becker et al., 2004; Grivennikov et al., 2009; Hedvat et al., 2009) and may therefore serve as a basis for preventive and therapeutic approaches. Altogether, cytokine production by immune and inflammatory cells is an important tumor promoting mechanism that provides malignant cells with a continuous supply of growth and survival signals in an initially hostile microenvironment. In most cases, tumor promoting cytokines act in a paracrine manner, yet several types of cancer cells produce their own cytokines, including IL-6, to achieve the same effect (Gao et al., 2007).

Inflammation and angiogenesis

Growth of large tumors requires an increased intratumoral blood supply. This is triggered by tumor hypoxia, which promotes angiogenesis and increases the probability of metastasis. In addition to hypoxia, tumor angiogenesis depends on recruitment of TAMs, which sense hypoxic signals and in turn produce chemokines and pro-angiogenic factors. Recruitment of TAM precursors is largely dependent on angiogenic mediators such as angiopoetin 2 and vascular endothelial growth factor (VEGF). Important pro-angiogenic genes, such as IL-8, CXCL1, CXCL8, VEGF and hypoxia inducible factor 1 alpha (HIF1□), are directly regulated by NF-□B, STAT3 and AP-1 in TAMs, MDSCs, and other cell types (Kujawski et al., 2008; Rius et al., 2008).

Under hypoxic conditions, HIF-1□stimulates expression of CXCL12, which activates and recruits endothelial cells in a CXCR4-dependent manner (Sica et al., 2008). Formation of new lymphatic vessels is regulated by VEGF-C and VEGF-D, whereas VEGF-A facilitates the recruitment of monocytes, which activate lymphoangiogenesis (Murdoch et al., 2008). VEGF-A produced by myeloid cells also inhibits pericyte maturation and endothelial coverage of newly formed blood vessels, and its conditional ablation accelerates tumorigenesis (Stockmann

et al., 2008). The recruitment of $Gr1^+$ myeloid cells (presumably MDSC and TAM precursors) into tumors, curtails the effects of anti-VEGF therapy, presumably bypassing the requirement for local VEGF production by cancer cells for recruitment of TAM precursors (Shojaei et al., 2007). As most growing tumors contain some areas of hypoxia, it is not clear whether hypoxia is the direct driver of tumor angiogenesis or whether hypoxic stimuli generate inflammatory signals that drive angiogenesis. Inactivation of NF-□B or STAT3, neutralization of CCL2 or CXCL12, or TAM depletion unequivocally result in disrupted angiogenesis and decreased tumor growth, underscoring the critical role of inflammatory mediators in tumor angiogenesis (Joyce and Pollard, 2009; Kujawski et al., 2008).

Target genes that mediate tumor promotion

Most of the genes that mediate the tumor promoting functions of NF-□B, STAT3, and AP-1 have not been fully defined and most likely the pro-tumorigenic effects of these transcription factors are exerted through multiple effectors. Some targets may be controlled by more than one transcription factor and may be more important in one cell type than in another. The expression of the anti-apoptotic proteins Bcl-2 and Bcl-X_L, for instance, are promoted by both NF-□B and STAT3 as are c-IAP1, c-IAP2, Mcl-1, c-FLIP, and survivin (Karin, 2006; Yu et al., 2007). Whereas Bcl-X_L may be the most prominent anti-apoptotic gene in enterocytes (Greten et al., 2004), c-FLIP seems to fulfill the same function in hepatocytes (Chang et al., 2006). Both NF-□B and STAT3 interfere with p53 synthesis and attenuate p53-mediated genomic surveillance, representing another potential tumor promoting mechanism (Colotta et al., 2009).

STAT3 controls expression of cyclins D1, D2 and B, as well as the proto-oncogene c-Myc, and through them it may stimulate cell proliferation (Bollrath et al., 2009; Yu et al., 2007). Although cyclin D and c-Myc are also thought to be regulated by NF-□B, inactivation of IKK□in enterocytes does not interfere with cell proliferation (Greten et al., 2004) and in Rastransformed keratinocytes (Zhang et al., 2004) or DEN-initiated hepatocytes (Maeda et al., 2005) NF-□B inhibition actually enhances cyclin D expression and cell proliferation. The AP-1 protein c-Jun cooperates with STAT3 in repression of Fas expression by tumor cells, thereby attenuating their sensitivity to instructive apoptosis (Eferl and Wagner, 2003). Additional NF-□B and STAT3 targets control cell and tissue resistance to stress and injury and include anti-microbial proteins (RegIII□, RegIII□, Tff3), heat shock proteins, and anti-oxidants, such as superoxide dismutase 2 (SOD2) and ferritin heavy chain (FHC) (Bollrath et al., 2009; Karin, 2006).

Lastly, another category of target genes that promote tumorigenesis are chemokines and cytokines that act in autocrine or paracrine manners to ensure the continuous recruitment of inflammatory cells into the tumor microenvironment. The perpetuation of chronic inflammation is largely achieved through positive feedback loops, which include inflammatory cells producing cytokines that induce chemokine synthesis in malignant and stromal cells leading to prolonged recruitment of inflammatory cells into the tumor microenvironment (Figure 3). TAMs, MDSCs, Tregs, and Th17 cells are the most critical immune cell subsets in this respect. Recruitment of myeloid cells is governed by multiple pathways, including CCL2-CCR2, CCL1-CXCR2, S100A proteins-RAGE, and IL-1-IL-1R interactions (Bonecchi et al., 2009). Signaling through CCR6 is critical for Th17 infiltration, whereas Treg cells are attracted mostly through CCR4 and CCR7 (Bonecchi et al., 2009). In some cases, the critical chemokines are not produced by cancer cells but are induced in tumor-associated fibroblasts upon interaction with carcinoma cells (Liao et al., 2009;Orimo et al., 2005;Orimo and Weinberg, 2006).

Inflammation and lymphoid malignancies

Chronic inflammatory conditions are also associated with lymphoid malignancies. An excellent example is provided by mucosa-associated lymphoid tissue (MALT) lymphomas, which occur in the context of chronic inflammation caused by infectious agents, such as *Helicobacter pylori* (the most commonly found gastric lymphoma), *Chlamydia psittacii* (ocular adnexal MALT lymphoma) and *Borrelia burgdorferi* (cutaneous MALT lymphoma) (Ferreri et al., 2009). Another example is Epstein-Barr virus (EBV), which is responsible for large B-cell lymphoma in immunocompromised patients, Burkitt's lymphoma, and Hodgkin's lymphoma (Ferreri et al., 2009).

It has been proposed that repeated antigenic stimulation, autoimmunity, and inflammation are risk factors for chronic lymphocytic leukemia (CLL), the most common hematopoietic malignancy that accounts for 30% of all leukemias (Chiorazzi et al., 2005). One mechanism through which such stimuli promote CLL development is induction of B cell activating factor (BAFF), a member of the TNF family, recently shown to accelerate development of CLL-like disease in mice (Enzler et al., 2009). Cytokines (such as IL-4 and VEGF), chemokines (such as SDF-1), and interactions with bone marrow stromal cells support CLL expansion and suppress apoptosis through upregulation of Bcl-2, survivin, and MCL-1 (Granziero et al., 2001; Pedersen et al., 2002). This occurs in lymph node pseudofollicles and bone marrow clusters where leukemic cells interact with components of the inflammatory microenvironment that support their survival. Another example for the role of inflammation in lymphoid malignancies are the lymphomas that appear in GM-CSF- and IFN□-deficient mice, which are caused by infections and regress upon treatment with antibiotics (Enzler et al., 2003).

A similar situation may occur in multiple myeloma. Through secretion of IL-6, IGF-1, VEGF, TNF-□, SDF-1 and BAFF, stromal elements promote the survival and migration of neoplastic plasma cells and also confer drug resistance (Kastritis et al., 2009). IL-6 is of particular importance, as it acts both in paracrine and autocrine manners and IL-6-deficient mice are resistant to induction of multiple myeloma (Hodge et al., 2005). Despite constitutive NF-□B activation, multiple myeloma remains dependent on extrinsic factors, and drugs targeting IL-6 are being evaluated in combination with the proteasome inhibitor bortezomib for the treatment of this malignancy (Kastritis et al., 2009).

Inflammation and metastasis

From a clinical perspective, metastasis is the most critical aspect of tumorigenesis, because over 90% of cancer mortality is caused by metastasis. Recent studies unambiguously show that metastasis requires close collaboration between cancer cells, immune and inflammatory cells, and stromal elements. The process of metastasis can be grossly divided into four major steps. The first step is represented by epithelial-mesenchymal transition, in which cancer cells acquire fibroblastoid characteristics that increase their motility and allow them to invade epithelial linings/basal membranes and reach efferent blood vessels or lymphatics (Kalluri and Weinberg, 2009). Loss of E-cadherin expression is envisioned as a key event in the epithelial-mesenchymal transition. In the second step, cancer cells intravasate into blood vessels and lymphatics. Inflammation may promote this through production of mediators that increase vascular permeability. This is followed by the third step in which metastasis initiating cells survive and travel throughout the circulation. It has been estimated that only about 0.01% of cancer cells that enter the circulation will eventually survive and give rise to micrometastases (Joyce and Pollard, 2009). Next, integrin-mediated arrest allows the extravasation of circulating cancer cells. Finally, single metastatic progenitors interact with immune, inflammatory, and stromal cells and start to proliferate (Polyak and Weinberg, 2009). Some of these cells may already be targeted to the pre-metastatic niche in response to tumor generated

inflammatory signals prior to the arrival of metastasis-initiating cancer cells (Kaplan et al., 2005). One of these inflammatory signals is the extracellular matrix component versican, which leads to macrophage activation and production of the metastasis promoting cytokine TNF- (Kim et al., 2009). However, it has been difficult to determine whether versican production by metastatic cancer cells conditions the future metastatic site prior to their arrival.

TGF is an anti-inflammatory cytokine produced by cancer cells, myeloid cells, and T lymphocytes. TGF signaling is an important regulator of the epithelial-mesenchymal transition and metastasis, and elevated TGF is often associated with poor prognosis (Yang and Weinberg, 2008). TGF activates SMAD transcription factors and MAPKs, which control expression of other regulators of the epithelial-mesenchymal transition, such as Slug (Yang and Weinberg, 2008). TGF however, also suppresses epithelial cell proliferation and early tumor growth, causing some tumors to acquire inactivating mutations in TGF signaling components (Yang and Weinberg, 2008). Despite the defects in TGF signaling, such tumors can still metastasize. These opposing effects of TGF at different stages of tumor development await mechanistic explanation. Disruption of TGF signaling in cancer cells also results in upregulation of the SDF1 (CXCL12)-CXCR4 and CXCL5-CXCR2 chemokine:chemokine receptor pairs and induces rapid recruitment of MDSCs that promote metastasis and dampen anti-tumor immune responses (Yang et al., 2008). Inactivation of TGF signaling was proposed to result in elevated local TGF concentrations that inhibit anti-tumor T cell responses and induce differentiation of tumor-promoting Th17 cells (Langowski et al., 2007).

Another critical regulator of the epithelial-mesenchymal transition is Snail, a repressor of E-cadherin transcription in epithelial cells. Recent findings suggest that Snail is stabilized in response to TNF- signaling, a process that is critical for cancer cell migration and metastasis (Wu et al., 2009b). Other mechanisms through which pro-inflammatory cytokines can affect the epithelial-mesenchymal transition is via STAT3-mediated induction of Twist transcription and NF-B-mediated induction of both Twist and Kiss (Yu et al., 2009), However, these mechanisms remain to be confirmed in vivo, and a recent report suggests that STAT3 is a negative regulator of adenoma-carcinoma transition in colon cancer (Musteanu et al., 2009).

Cancer cell invasion requires extensive proteolysis of the extracellular matrix at the invasive front. Inflammatory cells are important sources of proteases that degrade the extracellular matrix. In a model of invasive colon cancer, $CCR1^+$ myeloid cells, whose recruitment is driven by the chemokine CCL9 produced by cancer cells, promote invasiveness through secretion of the matrix metalloproteinases MMP2 and MMP9 (Kitamura et al., 2007). IL-1, TNF- and IL-6 promote MMP expression, invasiveness ,and metastasis via NF-B and STAT3 (Yu et al., 2007).

A different metastatic mechanism dependent on IKK operates in prostate and breast cancers. As these cancers progress, their malignant cells progressively accumulate activated IKK in their nuclei (Luo et al., 2007). In prostate cancer, accumulation of activated nuclear IKK correlates with reduced expression of maspin, an inhibitor of metastasis (Luo et al., 2007). IKK activation in metastatic prostate and mammary cancer cells is mediated by members of the TNF family, namely lymphotoxin and RANKL and its repressive effects on maspin transcription are NF-B independent (Luo et al., 2007). How these lymphocytes are recruited into progressing breast and prostate tumors is still unknown. Recruitment of such cells may be a consequence of tumor necrosis, but as mentioned above certain carcinomas actively secrete factors that upregulate fibronectin and cause migration of VEGF receptor 1 (VEGFR1)-positive hematopoietic progenitors to the pre-metastatic niche (Kaplan et al., 2005). However, the pre-metastatic niche concept is somewhat mysterious as it is not clear how primary tumor cells direct inflammatory cells to such sites.

Alternatively, a small number of metastatic cells can interact with and activate different myeloid cell types through secreted factors such as versican (Kim et al., 2009). Breast cancer cells use CSF1 and CXCL12 to induce the recruitment of TAMs, which in turn produce EGF receptor (EGFR) ligands (Joyce and Pollard, 2009). These cytokines may also mediate a physical interaction between TAMs and carcinoma cells (Condeelis and Pollard, 2006). TAMs can be also "programmed" by tumor infiltrating T cells, particularly Th17 cells (Wang et al., 2009) and Th2 cells (DeNardo et al., 2009). IL-13 and IL-4 produced by tumor infiltrating $CD4^+$ T cells stimulate the M1 to M2 transition of TAMs and thereby support pulmonary metastasis of mammary cancer cells (DeNardo et al., 2009). Depletion of TAMs (Joyce and Pollard, 2009) or $CD4^+$ T cells (DeNardo et al., 2009) dramatically reduces metastasis of mouse mammary cancer.

Once metastatic cells enter the circulation, they need to survive in suspension and resist detachment-induced cell death or anoikis. The survival of circulating cancer cells is affected by inflammatory mediators released by immune cells in response to cancer-derived or pathogen-derived stimuli (Kim et al., 2009; Luo et al., 2004). Some of these effects depend on activation of NF-□B in either inflammatory cells or in cancer cells. A variety of cytokines present in the tumor microenvironment, including TNF-□, IL-6, and epiregulin, can promote the survival of circulating metastatic seeds (Nguyen et al., 2009). In addition to NF-□B and STAT3 activation, some of these cytokines can physically link cancer cells to TAMs, allowing them to travel together throughout the circulation (Condeelis and Pollard, 2006). On the other hand, single metastatic cells, which are no longer present within an immunosuppressive environment, may be targeted again by immunosurveillance. Indeed, in some cases, infiltration of tumors by activated T cells decreases the rate of metastasis (Galon et al., 2006; Pages et al., 2005). The interaction of circulating cancer cells with platelets or macrophages may protect them from NK cell-mediated killing, thereby overcoming immunosurveillance (Palumbo et al., 2007).

Intravasation is regulated by prostaglandins (which are produced in a COX2-dependent manner and act on the epithelium), by cytokines (such as epiregulin, which increases cancer cell survival), and by MMPs (which clear the way for the latter to migrate into capillaries (Nguyen et al., 2009)). The migration of metastasis initiating cells is not random and is directed by chemokine gradients sensed via CXCR4, CCR4, CCR7, CCR9 and CCR10 (Bonecchi et al., 2009).

The journey of the circulating metastatic seed ends upon integrin-dependent arrest on the endothelium, followed by extravasation. Molecules like ANGPTL4, which is regulated by TGF□, facilitate extravasation into lungs by mediating contact between malignant and endothelial cells (Nguyen et al., 2009). Systemic inflammation enhances attachment of circulating cancer cells to hepatic sinusoids and this process is governed by neutrophil-dependent upregulation of adhesion molecules (McDonald et al., 2009). Several proinflammatory cytokines that are elevated in the circulation of cancer patients upregulate expression of adhesion molecules on the endothelium or in target organs and thereby increase the probability of metastatic cell attachment (Mantovani et al., 2008).

Immunity and tumorigenesis

As discussed above, in tumors that arise in the context of underlying inflammation or in advanced tumors containing inflammatory infiltrates, the net effect of the immune system (both innate and adaptive) is stimulation of tumor growth and progression. However, cancer cells represent an "altered self" and express "non-self" antigens in the context of stress and danger signals that can promote antigen presentation. Thus, even growing tumors may be subject to immunosurveillance and killing by activated T and NK cells (Dunn et al., 2004). It is likely

that immunosurveillance and tumor-promoting inflammation can coexist even in the same tumor (Bui and Schreiber, 2007) (Figure 4A).

According to the immunosurveillance hypothesis, NK cells and CTLs engage in tumor killing (via perforin, granzyme B, TRAIL or FasL dependent mechanisms), whereas Th1 (by virtue of IFN□production) and in some instances Th17 cells (via production of IL-17A) provide important help that boosts cytotoxic immunity (Dunn et al., 2006; Dunn et al., 2004; Martin-Orozco et al., 2009). On the other hand, Tregs suppress anti-tumor immune responses and are therefore pro-tumorigenic (Dunn et al., 2004). NKT cells can also be involved in surveillance of hematopoietic and chemically-induced tumors (Crowe et al., 2005; Smyth et al., 2000; Swann et al., 2009). Other critical components of this system are dendritic cells and macrophages, which present antigens and respond to danger and stress signals, as well as immunoregulatory and cytotoxic cytokines, such as type I IFN, IFN□, FasL, TRAIL, GM-CSF and IL-12 (Palucka et al., 2007; Smyth et al., 2006; Swann and Smyth, 2007).

The first experimental demonstration of tumor immunosurveillance came from analysis of Rag2-deficient mice, which lack mature lymphocytes. These mice show enhanced development of a variety of spontaneous cancers by 14-16 months of age (Shankaran et al., 2001). However, even in immunocompromised mice, tumor development occurs in their post-reproductive period, suggesting that the mammalian immune system is not subjected to substantial evolutionary pressure to improve tumor recognition and elimination. Yet, in virallyor bacterially-promoted cancers, the immune system provides considerable protection through its ability to recognize and eliminate microbes (Smyth et al., 2006). Inactivation of various components of the immunosurveillance system, such as perforin, granzyme, and interferon signaling, renders mice susceptible to tumorigenesis (Bui and Schreiber, 2007; Dunn et al., 2004). Mice lacking cytotoxic cytokines, such as membrane-bound forms of FasL or TRAIL also show enhanced development of sarcomas and other tumors (O' Reilly et al., 2009; Smyth et al., 2003).

More evidence for tumor immunosurveillance and immunoediting comes from the presence of tumor infiltrating lymphocytes (both T and B lymphocytes) that recognize tumor antigens and the favorable prognosis for some patients whose tumors display increased infiltration with activated T cells (Dunn et al., 2004). Such infiltration is even more noticeable in tumors that develop microsatellite instability or have a "mutator" phenotype and therefore express tumor antigens that exhibit greater differences from normal counterparts (Buckowitz et al., 2005; Guidoboni et al., 2001). Additional but indirect evidence for anti-tumor immunity includes various cases of spontaneous tumor regression accompanied by increased infiltration of activated cytotoxic cells and presence of antibodies and T cells that recognize tumor antigens (Swann and Smyth, 2007). The latter suggests that B and T lymphocytes have been activated by tumor-specific antigens but does not necessarily mean that these cells are responsible for tumor regression. Additional evidence is provided by the increased risk of lymphomas (of viral and non-viral etiology) and some solid tumors in immunosuppressed patients (Swann and Smyth, 2007).

Nonetheless, in the vast majority of established tumors the presence of tumor infiltrating lymphocytes is insufficient for curtailing tumor growth. Such considerations have given rise to a revised version of the immunosurveillance theory called immunoediting (Dunn et al., 2004; Smyth et al., 2006). According to this concept, cancer cells constantly edit and modulate the host anti-tumor immune response and the host immune response shapes tumor immunogenicity and clonal selection. During this process the balance between anti-tumor and tumor-promoting immunity can be tilted in favor of tumor growth. Before a tumor undergoes immune escape, it may be maintained at an "equilibrium" between tumor growth and immune destruction, and this may account for decades of tumor dormancy (Koebel et al., 2007). To tilt

the balance in its favor, it is proposed that the cancer cell edits its repertoire of tumor antigens towards lower immunogenicity and also re-shapes the tumor microenvironment to become immunosuppressive. Consistent with this hypothesis, cancers that have evolved in alymphocytic mice are more immunogenic than cancers grown in immunocompetent mice (Shankaran et al., 2001).

Therapy induced inflammation – friend or foe?

Surgery, chemotherapy, and radiation are currently the major options for cancer treatment. All three induce local or systemic inflammation triggered by tissue injury and cancer cell death. Surgery results in activation of infectionor stress-sensing pathways, whereas chemo- and radiotherapy kill cancer cells mostly through necrosis, a pro-inflammatory form of cell death (Vakkila and Lotze, 2004). Inflammatory mediators released by necrotic cells include danger associated molecular patterns (DAMPs) such as ATP, nucleic acids, heat shock proteins (Hsp70), HMGB-1, S100 calcium binding proteins, and the cytokine IL-1□. A key question is whether therapy-induced inflammation stimulates the regrowth of residual malignant cells or whether it improves the therapeutic outcome? (Figure 4B). In support of the first possibility, inhibition of autophagy in apoptosis-deficient tumors stimulates tumor growth through induction of necrosis and tumor-associated sterile inflammation (Degenhardt et al., 2006). Tumor growth may also be stimulated in response to hypoxia-induced necrosis in the tumor's core (Figure 4B). It has also been found that castration-induced death of androgen-dependent prostate cancer, despite resulting in initial tumor regression, triggers an inflammatory response that accelerates the re-growth of castration resistant cancer (Ammirante et al., 2010). Hence, inhibition of therapy-induced inflammation may improve the treatment of prostate cancer and provide the patient with several more years of tumor free survival.

However, in the case of more conventional chemotherapy, therapy-induced inflammation has been found to stimulate antigen presentation by tumor infiltrating dendritic cells and to induce production of cytokines that stimulate adaptive anti-tumor immunity (Apetoh et al., 2007a; Zhang et al., 2007) (Figure 4B). Curiously, the inflammatory trigger for this beneficial response is also the necrotic death of cancer cells, resulting in the release of HMG-B1 and ATP, which together activate TLR4 and the inflammasome to stimulate production of IL-1□, which is critical for adaptive anti-tumor immunity (Ghiringhelli et al., 2009). Interestingly, genetic polymorphisms in the TLR4 and P2X7 (the ATP receptor) loci affect the outcome of chemotherapy (Apetoh et al., 2007a; Apetoh et al., 2007b). What makes tumor necrosis either immunostimulatory or immunosuppressive (Vakkila and Lotze, 2004) is not yet clear. Furthermore, therapy-induced anti-tumor immunity is only seen with certain drugs, including etoposide, oxaliplatin, and doxorubicine but not with others (Apetoh et al., 2007a; Ghiringhelli et al., 2009). As these drugs can also kill infiltrating immune and hematopoietic stem cells, which are necessary for a functional immune response, effective therapy-induced anti-tumor immunity requires the use of small doses of chemotherapy to avoid immunosuppression. Conversely, by causing the death of tumor promoting immune/inflammatory cells, chemo- and radiotherapy may be used to destroy the tumor-promoting inflammatory microenvironment.

Anti-inflammatory drugs in cancer therapy

The findings described above provide an improved understanding of the molecular etiology of cancer and lay the foundations for the use of anti-inflammatory drugs in cancer prevention and therapy. One advantage of targeting the inflammatory microenvironment is that the normal genome of inflammatory/immune cells, which unlike the cancer cell genome, is not subject to mutational and epigenetic changes that result in drug resistance. However, in most cases, anti-inflammatory therapy is not cytocidal on its own and needs to be combined with more conventional therapies that kill cancer cells.

Despite such limitations, several anti-inflammatory drugs have been found to reduce tumor incidence when used as prophylactics, as well as slowing down progression and reducing mortality when used as therapeutics, particularly in the case of sporadic colon cancer (Gupta and Dubois, 2001). Such drugs include COX2 inhibitors, aspirin, and anti-inflammatory steroids, such as dexamethasone. In addition to its well-documented preventive effects in colon cancer, aspirin reduces the incidence of breast cancer (Gierach et al., 2008) and reduces prostate cancer risk, but only in individuals that carry a particular polymorphic allele at the lymphotoxin □locus, which specifies high lymphotoxin production (Liu et al., 2006). Such findings are of general importance because non-steroidal anti-inflammatory drugs (NSAID), such as aspirin, are not very specific and usually have side-effects that preclude their long-term administration except in high risk individuals. Thus, pre-screening for individuals with high cancer risk that are more likely to benefit from such preventive strategies should greatly improve the efficacy and utility of cancer prevention.

Tumor-promoting inflammation can be targeted in several different ways: 1) inhibition of signal transducers and transcription factors that mediate survival and growth of malignant cells in response to inflammatory cytokines; 2) sequestration of chemokines and cytokines that recruit and sustain inflammatory cells in the tumor microenvironment; 3) reducing (or augment) the inflammation that follows anti-cancer therapy; 4) depletion of immune and inflammatory cells that promote tumor development and progression, while sparing cell types and effector functions that support protective immune responses; 5) selective inhibition of tumor promoting cytokines without an effect on expression of anti-tumorigenic cytokines.

In a few cases, a therapy targeting inflammation may be effective as a single agent. For instance, constitutive NF-□B or STAT3 activation in certain lymphoid tumors suggests that inhibitors of these transcription factors can be used as cytocidal agents in such cancers. However in most cases such therapy is likely to be effective only in combination with more conventional approaches. Furthermore, as genotoxic therapies often lead to NF-□B activation in remaining malignant cells, it makes sense to combine genotoxic drugs with NF-□B inhibitors as a way to overcome drug resistance. However, prolonged NF-□B inhibition can result in a severe immune deficiency and may even lead to neutrophilia and greatly enhanced acute inflammation due to enhanced IL-1□secretion (Greten et al., 2007). Such complications as well as increased propensity for liver damage have hindered the clinical development of NF-□B and IKK□ inhibitors. Another attractive target is the STAT3 transcription factor and the signaling pathway that leads to its activation (Kortylewski et al., 2005; Yu et al., 2009). Several STAT3 and JAK2 inhibitors have been described and shown to inhibit the growth of various cancers that exhibit STAT3 activation (Hedvat et al., 2009; Lin et al., 2009). So far, none of the complications associated with NF-□B inhibition have been reported for STAT3 or JAK2 inhibitors.

Even fewer complications should be expected from drugs that inhibit receptor binding of pro-tumorigenic cytokines or chemokines. Several anti-cytokine drugs are already in use for the treatment of chronic inflammatory diseases or are under clinical development for such usage. Although cytokine inhibitors alone are unlikely to cause cancer cell death, several phase I/II clinical trials currently evaluate the efficacy of anti-IL-6 and anti-TNF-□drugs as single agents in various cancers (Balkwill, 2009). The effects obtained so far include disease stabilization and partial responses, but by-and-large the therapeutic effects are modest and underscore the necessity of evaluating such drugs in combination with conventional therapy. Anti-chemokine drugs are also being evaluated, including receptor antagonists and blocking antibodies, targeting CCR2, CCR4, and CXCR4 (Balkwill, 2009). IL-1 inhibition in multiple myeloma slows tumor growth and leads to a chronic disease state, thereby preventing progression to active myeloma (Lust et al., 2009).

Metastasis presents another important application and challenge for drugs that target tumor-associated inflammation. Recently, an anti-RANKL antibody, which was developed for the treatment of osteoporosis, has been found effective in inhibition of bone metastasis in prostate cancer (Hurst et al., 2009). Other experiments done in mice have shown that NF-□B inhibition in metastatic cancer cells or neutralization of TNF-□can convert inflammation promoted metastatic growth to inflammation-induced tumor regression, dependent on IFN-induced TRAIL expression (Luo et al., 2004). Such findings illustrate how manipulation of cytokine expression can be used to convert tumor- and metastasis-promoting inflammation to a strong anti-tumor response.

Conclusions and Prospective

Inflammation can affect every aspect of tumor development and progression as well as the response to therapy. In the past 10 years, we have learned a great deal about the different mechanisms by which cancer and inflammation intersect, and the time is right to translate much of the basic knowledge gained thus far and use it to add new armaments to the arsenal of cancer therapeutics. Only by targeting every single aspect of cancer biology, can we expect to make real gains in the fight against these currently incurable diseases. In addition to a combination of anti-inflammatory approaches that target the tumor microenvironment with more sophisticated and selective tumoricidal drugs, future therapies should also take notice of the natural genetic variation that affects inflammation and immunity. Such considerations are extremely important in the design of new preventive approaches to the reduction of cancer risk that need to be applied to large populations composed of relatively healthy individuals. Indeed, one of the major lessons learned from investigating the relationships between inflammation and cancer, is that most cancers are preventable. Prevention is a much better and more economic way to fight cancer than treating an already advanced and often intractable disease, as is done at the present.

Text Box: Inflammation and cancer-basic facts

1. Chronic inflammation increases cancer risk.
2. Subclinical, often undetectable, inflammation may be as important in increasing cancer risk (for instance, obesity-induced inflammation).
3. Various types of immune and inflammatory cells are frequently present within tumors.
4. Immune cells affect malignant cells through production of cytokines, chemokines, growth factors, prostaglandins and reactive oxygen and nitrogen species.
5. Inflammation impacts every single step of tumorigenesis, from initiation through tumor promotion, all the way to metastatic progression.
6. In developing tumors anti-tumorigenic and pro-tumorigenic immune and inflammatory mechanisms coexist, but if the tumor is not rejected, the pro-tumorigenic effect dominates.
7. Signaling pathways that mediate the pro-tumorigenic effects of inflammation are often subject to a feed-forward loop (for example, activation of NF-□B in immune cells induces production of cytokines that activate NF-□B in cancer cells to induce chemokines that attract more inflammatory cells into the tumor).
8. Certain immune and inflammatory components may be dispensable during one stage of tumorigenesis but absolutely critical in another stage.

Acknowledgments

We thank E. Koltsova for the help in figure preparation. This work was supported by Research Fellowship Award from Crohn's and Colitis Foundation of America (CCFA #1762) to S.G, by grants from the Deutsche Forschungsgemeinschaft, Deutsche Krebshilfe and the Association for International Cancer Research to F.R.G. and the National Institutes of Health and the American Association for Cancer Research to M.K., who is an American Cancer Society Research Professor.

REFERENCES

Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res 2009;15:425–430. [PubMed: 19147746]

Ammirante M, Luo J-L, Grivennikov S, Nedospasov S, Karin M. B cell-derived lymphotoxin promotes castration-resistant prostate cancer. Nature. 2010 in press.

Angel P, Imagawa M, Chiu R, Stein B, Imbra RJ, Rahmsdorf HJ, Jonat C, Herrlich P, Karin M. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell 1987;49:729–739. [PubMed: 3034432]

Apetoh L, Ghiringhelli F, Tesniere A, Criollo A, Ortiz C, Lidereau R, Mariette C, Chaput N, Mira JP, Delaloge S, et al. The interaction between HMGB1 and TLR4 dictates the outcome of anticancer chemotherapy and radiotherapy. Immunol Rev 2007a;220:47–59. [PubMed: 17979839]

Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, Mignot G, Maiuri MC, Ullrich E, Saulnier P, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007b;13:1050–1059. [PubMed: 17704786]

Aspord C, Pedroza-Gonzalez A, Gallegos M, Tindle S, Burton EC, Su D, Marches F, Banchereau J, Palucka AK. Breast cancer instructs dendritic cells to prime interleukin 13-secreting CD4+ T cells that facilitate tumor development. J Exp Med 2007;204:1037–1047. [PubMed: 17438063]

Balkwill F. Tumour necrosis factor and cancer. Nat Rev Cancer 2009;9:361–371. [PubMed: 19343034]

Becker C, Fantini MC, Schramm C, Lehr HA, Wirtz S, Nikolaev A, Burg J, Strand S, Kiesslich R, Huber S, et al. TGF-beta suppresses tumor progression in colon cancer by inhibition of IL-6 trans-signaling. Immunity 2004;21:491–501. [PubMed: 15485627]

Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, Thompson-Snipes L, Leach MW, Rennick D. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. J Clin Invest 1996;98:1010–1020. [PubMed: 8770874]

Bollrath J, Phesse TJ, von Burstin VA, Putoczki T, Bennecke M, Bateman T, Nebelsiek T, Lundgren-May T, Canli O, Schwitalla S, et al. gp130-mediated Stat3 activation in enterocytes regulates cell survival and cell-cycle progression during colitis-associated tumorigenesis. Cancer Cell 2009;15:91–102. [PubMed: 19185844]

Bonecchi R, Galliera E, Borroni EM, Corsi MM, Locati M, Mantovani A. Chemokines and chemokine receptors: an overview. Front Biosci 2009;14:540–551. [PubMed: 19273084]

Buckowitz A, Knaebel HP, Benner A, Blaker H, Gebert J, Kienle P, von Knebel Doeberitz M, Kloor M. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. Br J Cancer 2005;92:1746–1753. [PubMed: 15856045]

Bui JD, Schreiber RD. Cancer immunosurveillance, immunoediting and inflammation: independent or interdependent processes? Curr Opin Immunol 2007;19:203–208. [PubMed: 17292599]

Calle EE. Obesity and cancer. Br Med J 2007;335:1107–1108. [PubMed: 17986715]

Chang L, Kamata H, Solinas G, Luo JL, Maeda S, Venuprasad K, Liu YC, Karin M. The E3 ubiquitin ligase itch couples JNK activation to TNFa-induced cell death by inducing c-FLIP(L) turnover. Cell 2006;124:601–613. [PubMed: 16469705]

Chen X, Xu H, Yuan P, Fang F, Huss M, Vega VB, Wong E, Orlov YL, Zhang W, Jiang J, et al. Integration of external signaling pathways with the core transcriptional network in embryonic stem cells. Cell 2008;133:1106–1117. [PubMed: 18555785]

Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med 2005;352:804–815. [PubMed: 15728813]

Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. Carcinogenesis 2009;30:1073–1081. [PubMed: 19468060]

Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006;124:263–266. [PubMed: 16439202]

Cooper CS, Foster CS. Concepts of epigenetics in prostate cancer development. Br J Cancer 2009;100:240–245. [PubMed: 19002169]

Crowe NY, Coquet JM, Berzins SP, Kyparissoudis K, Keating R, Pellicci DG, Hayakawa Y, Godfrey DI, Smyth MJ. Differential antitumor immunity mediated by NKT cell subsets in vivo. J Exp Med 2005;202:1279–1288. [PubMed: 16275765]

de Martel C, Franceschi S. Infections and cancer: established associations and new hypotheses. Crit Rev Oncol Hematol 2009;70:183–194. [PubMed: 18805702]

De Santa F, Totaro MG, Prosperini E, Notarbartolo S, Testa G, Natoli G. The histone H3 lysine-27 demethylase Jmjd3 links inflammation to inhibition of polycomb-mediated gene silencing. Cell 2007;130:1083–1094. [PubMed: 17825402]

de Visser KE, Eichten A, Coussens LM. Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 2006;6:24–37. [PubMed: 16397525]

Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gelinas C, Fan Y, et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 2006;10:51–64. [PubMed: 16843265]

DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, Coussens LM. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell 2009;16:91–102. [PubMed: 19647220]

Dostert C, Petrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. Science 2008;320:674–677. [PubMed: 18403674]

Dougan M, Dranoff G. Immune therapy for cancer. Annu Rev Immunol 2009;27:83–117. [PubMed: 19007331]

Dunn GP, Koebel CM, Schreiber RD. Interferons, immunity and cancer immunoediting. Nat Rev Immunol 2006;6:836–848. [PubMed: 17063185]

Dunn GP, Old LJ, Schreiber RD. The immunobiology of cancer immunosurveillance and immunoediting. Immunity 2004;21:137–148. [PubMed: 15308095]

Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med 1986;315:1650–1659. [PubMed: 3537791]

Edwards RA, Witherspoon M, Wang K, Afrasiabi K, Pham T, Birnbaumer L, Lipkin SM. Epigenetic repression of DNA mismatch repair by inflammation and hypoxia in inflammatory bowel disease-associated colorectal cancer. Cancer Res 2009;69:6423–6429. [PubMed: 19638594]

Eferl R, Wagner EF. AP-1: a double-edged sword in tumorigenesis. Nat Rev Cancer 2003;3:859–868. [PubMed: 14668816]

Enzler T, Gillessen S, Manis JP, Ferguson D, Fleming J, Alt FW, Mihm M, Dranoff G. Deficiencies of GM-CSF and interferon gamma link inflammation and cancer. J Exp Med 2003;197:1213–1219. [PubMed: 12732663]

Enzler T, Kater AP, Zhang W, Widhopf GF 2nd, Chuang HY, Lee J, Avery E, Croce CM, Karin M, Kipps TJ. Chronic lymphocytic leukemia of E{micro}-TCL1 transgenic mice undergoes rapid cell turnover that can be offset by extrinsic CD257 to accelerate disease progression. Blood 2009;114:4469–4476. [PubMed: 19755673]

Erdman SE, Sohn JJ, Rao VP, Nambiar PR, Ge Z, Fox JG, Schauer DB. CD4+CD25+ regulatory lymphocytes induce regression of intestinal tumors in ApcMin/+ mice. Cancer Res 2005;65:3998–4004. [PubMed: 15899788]

Ernst M, Najdovska M, Grail D, Lundgren-May T, Buchert M, Tye H, Matthews VB, Armes J, Bhathal PS, Hughes NR, et al. STAT3 and STAT1 mediate IL-11-dependent and inflammation-associated gastric tumorigenesis in gp130 receptor mutant mice. J Clin Invest 2008;118:1727–1738. [PubMed: 18431520]

Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annu Rev Med 2000;51:245–270. [PubMed: 10774463]

Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759–767. [PubMed: 2188735]

Ferreri AJ, Ernberg I, Copie-Bergman C. Infectious agents and lymphoma development: molecular and clinical aspects. J Intern Med 2009;265:421–438. [PubMed: 19298458]

Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM. Polarization of tumor-associated neutrophil phenotype by TGF-beta: “N1” versus “N2” TAN. Cancer Cell 2009;16:183–194. [PubMed: 19732719]

Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9:162–174. [PubMed: 19197294]

Gallimore AM, Simon AK. Positive and negative influences of regulatory T cells on tumour immunity. Oncogene 2008;27:5886–5893. [PubMed: 18836469]

Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–1964. [PubMed: 17008531]

Gao SP, Mark KG, Leslie K, Pao W, Motoi N, Gerald WL, Travis WD, Bornmann W, Veach D, Clarkson B, et al. Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J Clin Invest 2007;117:3846–3856. [PubMed: 18060032]

Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, Vermaelen K, Panaretakis T, Mignot G, Ullrich E, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med 2009;15:1170–1178. [PubMed: 19767732]

Gierach GL, Lacey JV Jr. Schatzkin A, Leitzmann MF, Richesson D, Hollenbeck AR, Brinton LA. Nonsteroidal anti-inflammatory drugs and breast cancer risk in the National Institutes of Health-AARP Diet and Health Study. Breast Cancer Res 2008;10:R38. [PubMed: 18447943]

Granziero L, Ghia P, Circosta P, Gottardi D, Strola G, Geuna M, Montagna L, Piccoli P, Chilosi M, Caligaris-Cappio F. Survivin is expressed on CD40 stimulation and interfaces proliferation and apoptosis in B-cell chronic lymphocytic leukemia. Blood 2001;97:2777–2783. [PubMed: 11313271]

Greten FR, Arkan MC, Bollrath J, Hsu LC, Goode J, Miething C, Goktuna SI, Neuenhahn M, Fierer J, Paxian S, et al. NF-kappaB is a negative regulator of IL-1beta secretion as revealed by genetic and pharmacological inhibition of IKKbeta. Cell 2007;130:918–931. [PubMed: 17803913]

Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKb links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 2004;118:285–296. [PubMed: 15294155]

Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 2009;15:103–113. [PubMed: 19185845]

Grivennikov S, Karin M. Dangerous liasons: STAT3 and NF-□B collaboration and crosstalk in cancer. Cytokine Growth Factor Rev. 2009 in press.

Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, Dubus P, Sandgren EP, Barbacid M. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell 2007;11:291–302. [PubMed: 17349585]

Guidoboni M, Gafa R, Viel A, Doglioni C, Russo A, Santini A, Del Tin L, Macri E, Lanza G, Boiocchi M, et al. Microsatellite instability and high content of activated cytotoxic lymphocytes identify colon cancer patients with a favorable prognosis. Am J Pathol 2001;159:297–304. [PubMed: 11438476]

Gupta RA, Dubois RN. Colorectal cancer prevention and treatment by inhibition of cyclooxygenase-2. Nat Rev Cancer 2001;1:11–21. [PubMed: 11900248]

Hahn MA, Hahn T, Lee DH, Esworthy RS, Kim BW, Riggs AD, Chu FF, Pfeifer GP. Methylation of polycomb target genes in intestinal cancer is mediated by inflammation. Cancer Res 2008;68:10280–10289. [PubMed: 19074896]

Hanada T, Kobayashi T, Chinen T, Saeki K, Takaki H, Koga K, Minoda Y, Sanada T, Yoshioka T, Mimata H, et al. IFNgamma-dependent, spontaneous development of colorectal carcinomas in SOCS1-deficient mice. J Exp Med 2006;203:1391–1397. [PubMed: 16717119]

Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70. [PubMed: 10647931]

Haybaeck J, Zeller N, Wolf MJ, Weber A, Wagner U, Kurrer MO, Bremer J, Iezzi G, Graf R, Clavien PA, et al. A lymphotoxin-driven pathway to hepatocellular carcinoma. Cancer Cell 2009;16:295–308. [PubMed: 19800575]

Hedvat M, Huszar D, Herrmann A, Gozgit JM, Schroeder A, Sheehy A, Buettner R, Proia D, Kowolik CM, Xin H, et al. The JAK2 Inhibitor AZD1480 Potently Blocks Stat3 Signaling and Oncogenesis in Solid Tumors. Cancer Cell 2009;16:487–497. [PubMed: 19962667]

Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. Eur J Cancer 2005;41:2502–2512. [PubMed: 16199153]

Hurst LC, Badalamente MA, Hentz VR, Hotchkiss RN, Kaplan FT, Meals RA, Smith TM, Rodzvilla J. Injectable collagenase clostridium histolyticum for Dupuytren's contracture. N Engl J Med 2009;361:968–979. [PubMed: 19726771]

Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276–285. [PubMed: 12671666]

Joyce JA, Pollard JW. Microenvironmental regulation of metastasis. Nat Rev Cancer 2009;9:239–252. [PubMed: 19279573]

Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119:1420–1428. [PubMed: 19487818]

Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, MacDonald DD, Jin DK, Shido K, Kerns SA, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 2005;438:820–827. [PubMed: 16341007]

Karin M. Nuclear factor-kappaB in cancer development and progression. Nature 2006;441:431–436. [PubMed: 16724054]

Kastritis E, Palumbo A, Dimopoulos MA. Treatment of relapsed/refractory multiple myeloma. Semin Hematol 2009;46:143–157. [PubMed: 19389498]

Khasawneh J, Schulz MD, Walch A, Rozman J, de Angelis M. Hrabe, Klingenspor M, Buck A, Schwaiger M, Saur D, Schmid RM, et al. Inflammation and mitochondrial fatty acid beta-oxidation link obesity to early tumor promotion. Proc Natl Acad Sci U S A 2009;106:3354–3359. [PubMed: 19208810]

Kim S, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y, Luo JL, Karin M. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. Nature 2009;457:102–106. [PubMed: 19122641]

Kitamura T, Kometani K, Hashida H, Matsunaga A, Miyoshi H, Hosogi H, Aoki M, Oshima M, Hattori M, Takabayashi A, et al. SMAD4-deficient intestinal tumors recruit CCR1+ myeloid cells that promote invasion. Nat Genet 2007;39:467–475. [PubMed: 17369830]

Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, Smyth MJ, Schreiber RD. Adaptive immunity maintains occult cancer in an equilibrium state. Nature 2007;450:903–907. [PubMed: 18026089]

Kohrt HE, Nouri N, Nowels K, Johnson D, Holmes S, Lee PP. Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. PLoS Med 2005;2:e284. [PubMed: 16124834]

Kortylewski M, Kujawski M, Wang T, Wei S, Zhang S, Pilon-Thomas S, Niu G, Kay H, Mule J, Kerr WG, et al. Inhibiting Stat3 signaling in the hematopoietic system elicits multicomponent antitumor immunity. Nat Med 2005;11:1314–1321. [PubMed: 16288283]

Kortylewski M, Xin H, Kujawski M, Lee H, Liu Y, Harris T, Drake C, Pardoll D, Yu H. Regulation of the IL-23 and IL-12 balance by Stat3 signaling in the tumor microenvironment. Cancer Cell 2009;15:114–123. [PubMed: 19185846]

Kraus S, Arber N. Inflammation and colorectal cancer. Curr Opin Pharmacol 2009;9:405–410. [PubMed: 19589728]

Kujawski M, Kortylewski M, Lee H, Herrmann A, Kay H, Yu H. Stat3 mediates myeloid cell-dependent tumor angiogenesis in mice. J Clin Invest 2008;118:3367–3377. [PubMed: 18776941]

Laghi L, Bianchi P, Miranda E, Balladore E, Pacetti V, Grizzi F, Allavena P, Torri V, Repici A, Santoro A, et al. CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastasis: a longitudinal study. Lancet Oncol 2009;10:877–884. [PubMed: 19656725]

Langowski JL, Kastelein RA, Oft M. Swords into plowshares: IL-23 repurposes tumor immune surveillance. Trends Immunol 2007;28:207–212. [PubMed: 17395538]

Langowski JL, Zhang X, Wu L, Mattson JD, Chen T, Smith K, Basham B, McClanahan T, Kastelein RA, Oft M. IL-23 promotes tumour incidence and growth. Nature 2006;442:461–465. [PubMed: 16688182]

Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res 2006;66:605–612. [PubMed: 16423985]

Liao D, Luo Y, Markowitz D, Xiang R, Reisfeld RA. Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. PLoS One 2009;4:e7965. [PubMed: 19956757]

Lin L, Amin R, Gallicano GI, Glasgow E, Jogunoori W, Jessup JM, Zasloff M, Marshall JL, Shetty K, Johnson L, et al. The STAT3 inhibitor NSC 74859 is effective in hepatocellular cancers with disrupted TGF-beta signaling. Oncogene 2009;28:961–972. [PubMed: 19137011]

Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 2007;117:1175–1183. [PubMed: 17476347]

Liu X, Plummer SJ, Nock NL, Casey G, Witte JS. Nonsteroidal antiinflammatory drugs and decreased risk of advanced prostate cancer: modification by lymphotoxin alpha. Am J Epidemiol 2006;164:984–989. [PubMed: 16931544]

Luo JL, Maeda S, Hsu LC, Yagita H, Karin M. Inhibition of NF-kappaB in cancer cells converts inflammation-induced tumor growth mediated by TNFalpha to TRAIL-mediated tumor regression. Cancer Cell 2004;6:297–305. [PubMed: 15380520]

Luo JL, Tan W, Ricono JM, Korchynskyi O, Zhang M, Gonias SL, Cheresh DA, Karin M. Nuclear cytokine-activated IKKalpha controls prostate cancer metastasis by repressing Maspin. Nature 2007;446:690–694. [PubMed: 17377533]

Lust JA, Lacy MQ, Zeldenrust SR, Dispenzieri A, Gertz MA, Witzig TE, Kumar S, Hayman SR, Russell SJ, Buadi FK, et al. Induction of a chronic disease state in patients with smoldering or indolent multiple myeloma by targeting interleukin 1{beta}-induced interleukin 6 production and the myeloma proliferative component. Mayo Clin Proc 2009;84:114–122. [PubMed: 19181644]

Maeda S, Kamata H, Luo JL, Leffert H, Karin M. IKKb couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 2005;121:977–990. [PubMed: 15989949]

Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436–444. [PubMed: 18650914]

Martin-Orozco N, Muranski P, Chung Y, Yang XO, Yamazaki T, Lu S, Hwu P, Restifo NP, Overwijk WW, Dong C. T helper 17 cells promote cytotoxic T cell activation in tumor immunity. Immunity 2009;31:787–798. [PubMed: 19879162]

McDonald B, Spicer J, Giannais B, Fallavollita L, Brodt P, Ferri LE. Systemic inflammation increases cancer cell adhesion to hepatic sinusoids by neutrophil mediated mechanisms. Int J Cancer 2009;125:1298–1305. [PubMed: 19431213]

Meira LB, Bugni JM, Green SL, Lee CW, Pang B, Borenshtein D, Rickman BH, Rogers AB, Moroski-Erkul CA, McFaline JL, et al. DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. J Clin Invest 2008;118:2516–2525. [PubMed: 18521188]

Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, Holdsworth H, Turner L, Rollins B, Pasparakis M, et al. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med 1999;5:828–831. [PubMed: 10395330]

Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer 2008;8:618–631. [PubMed: 18633355]

Musteanu M, Blaas L, Mair M, Schlederer M, Bilban M, Tauber S, Esterbauer H, Mueller M, Casanova E, Kenner L, et al. Stat3 is a negative regulator of intestinal tumor progression in ApcMin mice. Gastroenterology. 2009 in press.

Naugler WE, Sakurai T, Kim S, Maeda S, Kim K, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007;317:121–124. [PubMed: 17615358]

Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer 2009;9:274–284. [PubMed: 19308067]

Nickoloff BJ, Ben-Neriah Y, Pikarsky E. Inflammation and cancer: is the link as simple as we think? J Invest Dermatol 2005;124:x–xiv. [PubMed: 15955081]

O' Reilly L, Tai L, Lee L, Kruse EA, Grabow S, Fairlie WD, Haynes NM, Tarlinton DM, Zhang JG, Belz GT, et al. Membrane-bound Fas ligand only is essential for Fas-induced apoptosis. Nature 2009;461:659–663. [PubMed: 19794494]

Oguma K, Oshima H, Aoki M, Uchio R, Naka K, Nakamura S, Hirao A, Saya H, Taketo MM, Oshima M. Activated macrophages promote Wnt signalling through tumour necrosis factor-alpha in gastric tumour cells. EMBO J 2008;27:1671–1681. [PubMed: 18511911]

Okayasu I, Ohkusa T, Kajiura K, Kanno J, Sakamoto S. Promotion of colorectal neoplasia in experimental murine ulcerative colitis. Gut 1996;39:87–92. [PubMed: 8881816]

Okazaki IM, Kotani A, Honjo T. Role of AID in tumorigenesis. Adv Immunol 2007;94:245–273. [PubMed: 17560277]

Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005;121:335–348. [PubMed: 15882617]

Orimo A, Weinberg RA. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. Cell Cycle 2006;5:1597–1601. [PubMed: 16880743]

Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, et al. Effector memory T cells, early metastasis, and survival in colorectal cancer. N Engl J Med 2005;353:2654–2666. [PubMed: 16371631]

Palucka AK, Ueno H, Fay JW, Banchereau J. Taming cancer by inducing immunity via dendritic cells. Immunol Rev 2007;220:129–150. [PubMed: 17979844]

Palumbo JS, Talmage KE, Massari JV, La Jeunesse CM, Flick MJ, Kombrinck KW, Hu Z, Barney KA, Degen JL. Tumor cell-associated tissue factor and circulating hemostatic factors cooperate to increase metastatic potential through natural killer cell-dependent and-independent mechanisms. Blood 2007;110:133–141. [PubMed: 17371949]

Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer R, Osterreicher C, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell. 2010 in press.

Pedersen IM, Kitada S, Leoni LM, Zapata JM, Karras JG, Tsukada N, Kipps TJ, Choi YS, Bennett F, Reed JC. Protection of CLL B cells by a follicular dendritic cell line is dependent on induction of Mcl-1. Blood 2002;100:1795–1801. [PubMed: 12176902]

Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y. NF-kB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461–466. [PubMed: 15329734]

Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer 2009;9:265–273. [PubMed: 19262571]

Popivanova BK, Kitamura K, Wu Y, Kondo T, Kagaya T, Kaneko S, Oshima M, Fujii C, Mukaida N. Blocking TNF-alpha in mice reduces colorectal carcinogenesis associated with chronic colitis. J Clin Invest 2008;118:560–570. [PubMed: 18219394]

Punturieri A, Szabo E, Croxton TL, Shapiro SD, Dubinett SM. Lung cancer and chronic obstructive pulmonary disease: needs and opportunities for integrated research. J Natl Cancer Inst 2009;101:554–559. [PubMed: 19351920]

Rakoff-Nahoum S, Medzhitov R. Toll-like receptors and cancer. Nat Rev Cancer 2009;9:57–63. [PubMed: 19052556]

Rius J, Guma M, Schachtrup C, Akassoglou K, Zinkernagel AS, Nizet V, Johnson RS, Haddad GG, Karin M. NF-kappaB links innate immunity to the hypoxic response through transcriptional regulation of HIF-1alpha. Nature 2008;453:807–811. [PubMed: 18432192]

Roberts SJ, Ng BY, Filler RB, Lewis J, Glusac EJ, Hayday AC, Tigelaar RE, Girardi M. Characterizing tumor-promoting T cells in chemically induced cutaneous carcinogenesis. Proc Natl Acad Sci U S A 2007;104:6770–6775. [PubMed: 17412837]

Rodier F, Coppe JP, Patil CK, Hoeijmakers WA, Munoz DP, Raza SR, Freund A, Campeau E, Davalos AR, Campisi J. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 2009;11:973–979. [PubMed: 19597488]

Ruzankina Y, Schoppy DW, Asare A, Clark CE, Vonderheide RH, Brown EJ. Tissue regenerative delays and synthetic lethality in adult mice after combined deletion of Atr and Trp53. Nat Genet 2009;41:1144–1149. [PubMed: 19718024]

Sakurai T, He G, Matsuzawa A, Yu GY, Maeda S, Hardiman G, Karin M. Hepatocyte necrosis induced by oxidative stress and IL-1 alpha release mediate carcinogen-induced compensatory proliferation and liver tumorigenesis. Cancer Cell 2008;14:156–165. [PubMed: 18691550]

Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, Schreiber RD. IFNg and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature 2001;410:1107–1111. [PubMed: 11323675]

Shibata W, Takaishi S, Muthupalani S, Pritchard DM, Whary MT, Rogers AB, Fox JG, Betz KS, Kaestner KH, Karin M, et al. Conditional deletion of IkappaB-kinase beta (IKKbeta) accelerates Helicobacter-dependent gastric apoptosis, proliferation and preneoplasia. Gastroenterology. 2009 in press.

Shime H, Yabu M, Akazawa T, Kodama K, Matsumoto M, Seya T, Inoue N. Tumor-secreted lactic acid promotes IL-23/IL-17 proinflammatory pathway. J Immunol 2008;180:7175–7183. [PubMed: 18490716]

Shojaei F, Wu X, Malik AK, Zhong C, Baldwin ME, Schanz S, Fuh G, Gerber HP, Ferrara N. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. Nat Biotechnol 2007;25:911–920. [PubMed: 17664940]

Sica A, Allavena P, Mantovani A. Cancer related inflammation: the macrophage connection. Cancer Lett 2008;267:204–215. [PubMed: 18448242]

Sieweke MH, Thompson NL, Sporn MB, Bissell MJ. Mediation of wound-related Rous sarcoma virus tumorigenesis by TGF-beta. Science 1990;248:1656–1660. [PubMed: 2163544]

Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol 2006;90:1–50. [PubMed: 16730260]

Smyth MJ, Takeda K, Hayakawa Y, Peschon JJ, van den Brink MR, Yagita H. Nature's TRAIL--on a path to cancer immunotherapy. Immunity 2003;18:1–6. [PubMed: 12530970]

Smyth MJ, Thia KY, Street SE, Cretney E, Trapani JA, Taniguchi M, Kawano T, Pelikan SB, Crowe NY, Godfrey DI. Differential tumor surveillance by natural killer (NK) and NKT cells. J Exp Med 2000;191:661–668. [PubMed: 10684858]

Soucek L, Lawlor ER, Soto D, Shchors K, Swigart LB, Evan GI. Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. Nat Med 2007;13:1211–1218. [PubMed: 17906636]

Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. Cancer Cell 2004;6:447–458. [PubMed: 15542429]

Stockmann C, Doedens A, Weidemann A, Zhang N, Takeda N, Greenberg JI, Cheresh DA, Johnson RS. Deletion of vascular endothelial growth factor in myeloid cells accelerates tumorigenesis. Nature 2008;456:814–818. [PubMed: 18997773]

Strid J, Roberts SJ, Filler RB, Lewis JM, Kwong BY, Schpero W, Kaplan DH, Hayday AC, Girardi M. Acute upregulation of an NKG2D ligand promotes rapid reorganization of a local immune compartment with pleiotropic effects on carcinogenesis. Nat Immunol 2008;9:146–154. [PubMed: 18176566]

Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest 2007;117:1137–1146. [PubMed: 17476343]

Swann JB, Uldrich AP, van Dommelen S, Sharkey J, Murray WK, Godfrey DI, Smyth MJ. Type I natural killer T cells suppress tumors caused by p53 loss in mice. Blood 2009;113:6382–6385. [PubMed: 19234138]

Swann JB, Vesely MD, Silva A, Sharkey J, Akira S, Schreiber RD, Smyth MJ. Demonstration of inflammation-induced cancer and cancer immunoediting during primary tumorigenesis. Proc Natl Acad Sci U S A 2008;105:652–656. [PubMed: 18178624]

Takahashi H, Ogata H, Nishigaki R, Broide DH, Karin M. Tobacco smoke promotes lung tumorigenesis by triggering IKKb and JNK1 dependent inflammation. Cancer Cell. 2010 in press.

Takai A, Toyoshima T, Uemura M, Kitawaki Y, Marusawa H, Hiai H, Yamada S, Okazaki IM, Honjo T, Chiba T, et al. A novel mouse model of hepatocarcinogenesis triggered by AID causing deleterious p53 mutations. Oncogene 2009;28:469–478. [PubMed: 18997814]

Trinchieri G, Pflanz S, Kastelein RA. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. Immunity 2003;19:641–644. [PubMed: 14614851]

Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, Betz KS, Penz-Oesterreicher M, Bjorkdahl O, Fox JG, et al. Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. Cancer Cell 2008;14:408–419. [PubMed: 18977329]

Tuncman G, Hirosumi J, Solinas G, Chang L, Karin M, Hotamisligil GS. Functional in vivo interactions between JNK1 and JNK2 isoforms in obesity and insulin resistance. Proc Natl Acad Sci U S A 2006;103:10741–10746. [PubMed: 16818881]

Umar S, Sarkar S, Wang Y, Singh P. Functional Cross-talk between {beta}-Catenin and NF{kappa}B Signaling Pathways in Colonic Crypts of Mice in Response to Progastrin. J Biol Chem 2009;284:22274–22284. [PubMed: 19497850]

Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. Nat Rev Immunol 2004;4:641–648. [PubMed: 15286730]

Waldner MJ, Neurath MF. Colitis-associated cancer: the role of T cells in tumor development. Semin Immunopathol 2009;31:249–256. [PubMed: 19495757]

Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. J Exp Med 2009;206:1457–1464. [PubMed: 19564351]

Wong VW, Yu J, Cheng AS, Wong GL, Chan HY, Chu ES, Ng EK, Chan FK, Sung JJ, Chan HL. High serum interleukin-6 level predicts future hepatocellular carcinoma development in patients with chronic hepatitis B. Int J Cancer 2009;124:2766–2770. [PubMed: 19267406]

Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 2009a;15:1016–1022. [PubMed: 19701202]

Wu Y, Deng J, Rychahou PG, Qiu S, Evers BM, Zhou BP. Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion. Cancer Cell 2009b;15:416–428. [PubMed: 19411070]

Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 2008;14:818–829. [PubMed: 18539112]

Yang L, Huang J, Ren X, Gorska AE, Chytil A, Aakre M, Carbone DP, Matrisian LM, Richmond A, Lin PC, et al. Abrogation of TGF beta signaling in mammary carcinomas recruits Gr-1+CD11b+ myeloid cells that promote metastasis. Cancer Cell 2008;13:23–35. [PubMed: 18167337]

Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 2007;7:41–51. [PubMed: 17186030]

Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer 2009;9:798–809. [PubMed: 19851315]

Zhang B, Bowerman NA, Salama JK, Schmidt H, Spiotto MT, Schietinger A, Yu P, Fu YX, Weichselbaum RR, Rowley DA, et al. Induced sensitization of tumor stroma leads to eradication of established cancer by T cells. J Exp Med 2007;204:49–55. [PubMed: 17210731]

Zhang JY, Green CL, Tao S, Khavari PA. NF-kappaB RelA opposes epidermal proliferation driven by TNFR1 and JNK. Genes Dev 2004;18:17–22. [PubMed: 14724177]

Zheng L, Dai H, Zhou M, Li M, Singh P, Qiu J, Tsark W, Huang Q, Kernstine K, Zhang X, et al. Fen1 mutations result in autoimmunity, chronic inflammation and cancers. Nat Med 2007;13:812–819. [PubMed: 17589521]

Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nat Rev Immunol 2008;8:59–73. [PubMed: 18097448]

Zong WX, Thompson CB. Necrotic death as a cell fate. Genes Dev 2006;20:1–15. [PubMed: 16391229]

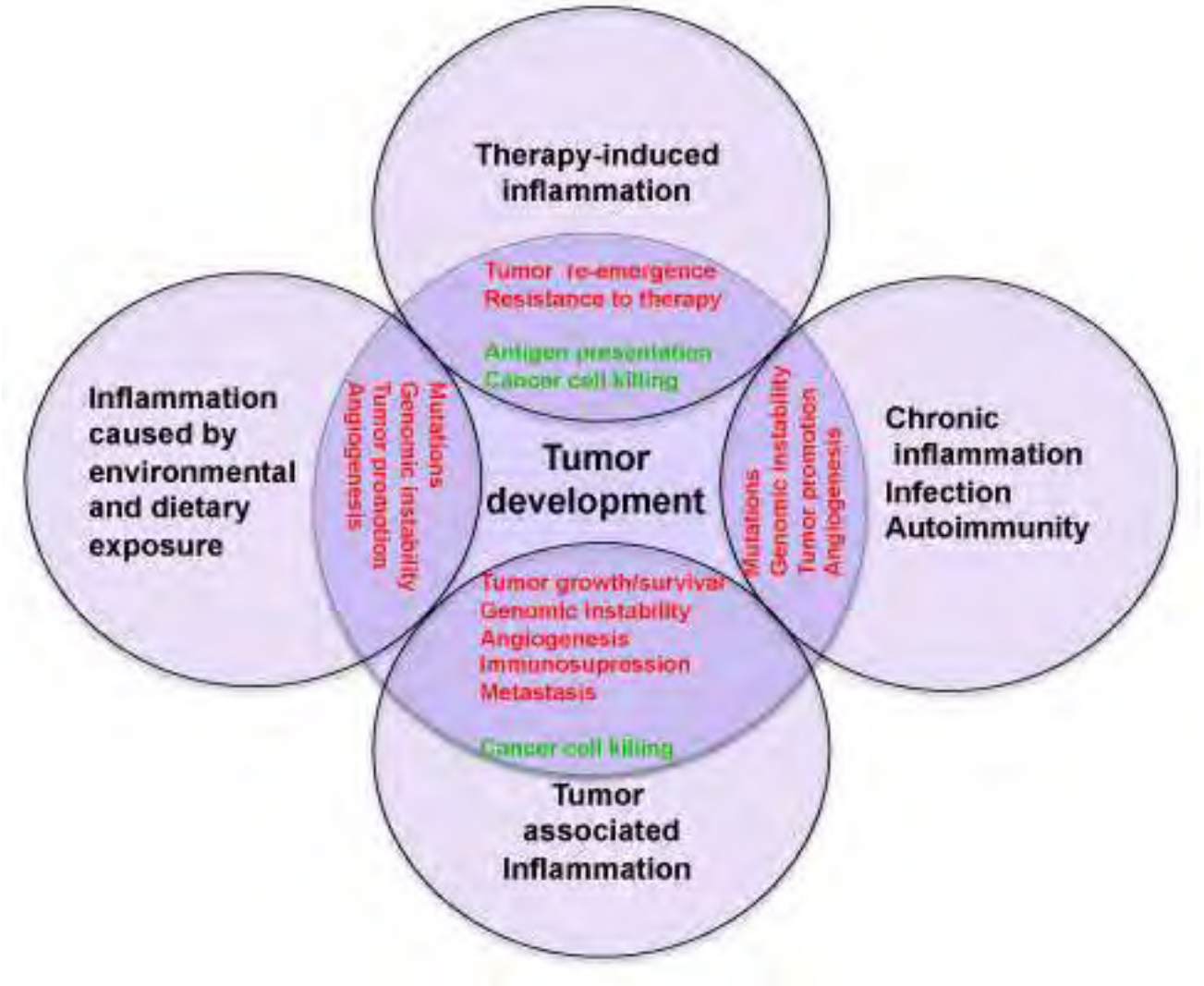

Figure 1.

Types of inflammation in tumorigenesis and cancer.

Chronic inflammation associated with infections or autoimmune disease precedes tumor development and can contribute to it through induction of oncogenic mutations, genomic instability, early tumor promotion, and enhanced angiogenesis. Prolonged exposure to environmental irritants or obesity can also result in low-grade chronic inflammation that precedes tumor development and contributes to it through the mechanisms mentioned above. Tumor-associated inflammation goes hand in hand with tumor development. This inflammatory response can enhance neo-angiogenesis, promote tumor progression and metastatic spread, cause local immunosuppression, and further augment genomic instability. Cancer therapy can also trigger an inflammatory response by causing trauma, necrosis, and tissue injury that stimulate tumor re-emergence and resistance to therapy. However, in some cases, therapy-induced inflammation can enhance antigen presentation, leading to immune-mediated tumor eradication. Tumor promoting mechanisms are in red and anti-tumorigenic mechanisms are in green.

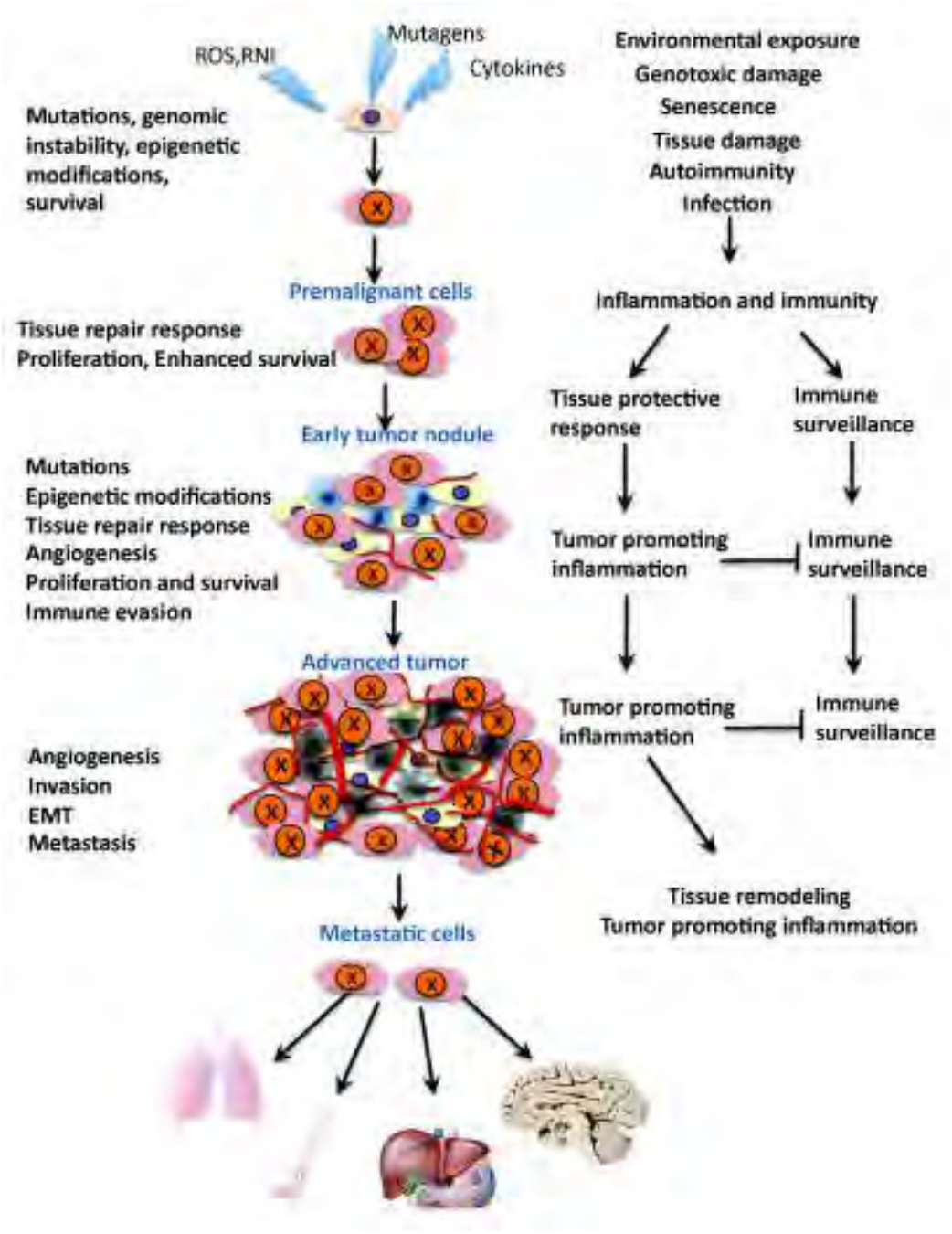

Figure 2.

The multifaceted role of inflammation in cancer

Inflammation acts at all stages of tumorigenesis. It may contribute to tumor initiation through mutations, genomic instability, and epigenetic modifications. Inflammation activates tissue repair responses, induces proliferation of premalignant cells, and enhances their survival. Inflammation also stimulates angiogenesis, causes localized immunosuppression, and promotes the formation of a hospitable microenvironment in which pre-malignant cells can survive, expand, and accumulate additional mutations and epigenetic changes. Eventually, inflammation also promotes metastatic spread. Mutated cells are marked with “X”. Yellow - stromal cells, Brown - malignant cells, Red - blood vessels, Blue - immune and inflammatory

cells. Epithelial-mesenchymal transition, EMT; reactive oxygen species, ROS; reactive nitrogen intermediates (RNI)

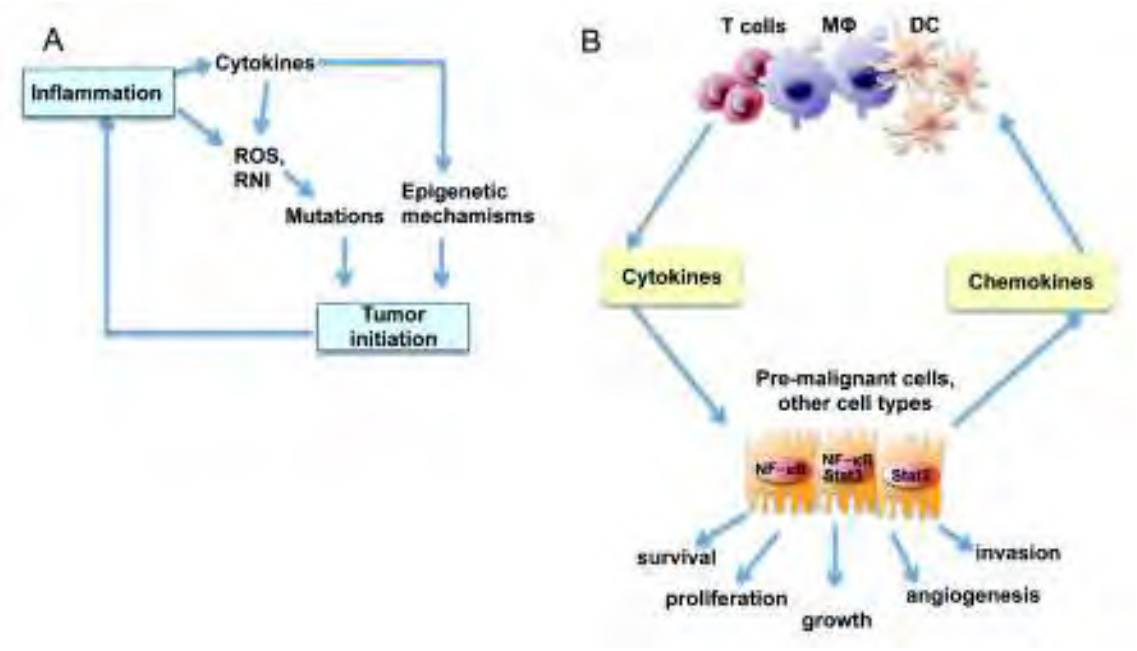

Figure 3.

Role of inflammation in tumor initiation and promotion

A) Tumor initiation. Reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) produced by inflammatory cells may cause mutations in neighboring epithelial cells. Also, cytokines produced by inflammatory cells can elevate intracellular ROS and RNI in pre-malignant cells. In addition, inflammation can result in epigenetic changes that favor tumor initiation. Tumor-associated inflammation contributes to further ROS, RNI and cytokine production.

B) Tumor promotion. Cytokines produced by tumor infiltrating immune cells activate key transcription factors, such as NF-□B or STAT3, in pre-malignant cells to control numerous pro-tumorigenic processes, including survival, proliferation, growth, angiogenesis, and invasion. As parts of positive feed-forward loops, NF-□B and STAT3 induce production of chemokines that attract additional immune/inflammatory cells to sustain tumor-associated inflammation.

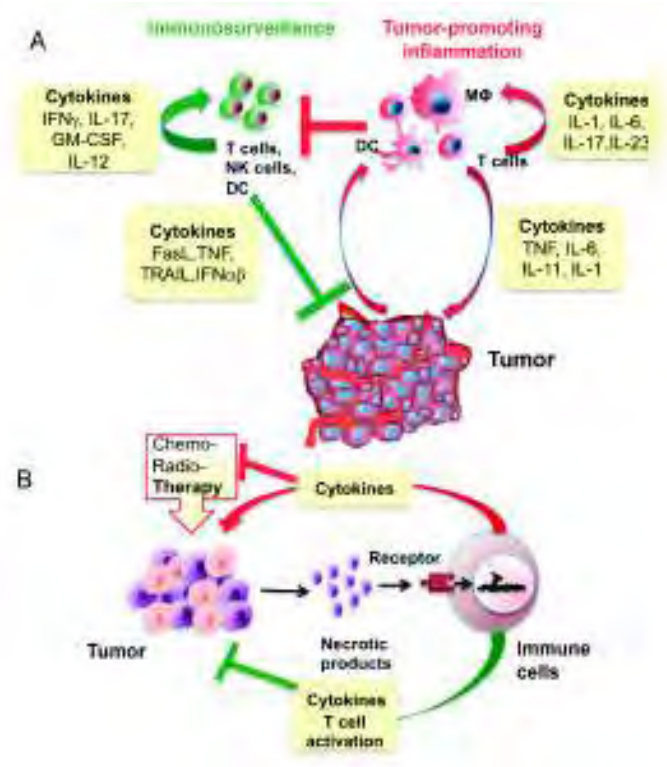

Figure 4.

Immunosurveillance, tumor-promoting and therapy-induced inflammation.

A) Balance between immunosurveillance and tumor promoting inflammation in the tumor microenvironment. Tumor promoting cytokines act on immune and malignant cells to tilt the balance toward tumor promotion. Tumor promoting immunity dampens immunosurveillance, which otherwise inhibits tumor growth. B) Therapy-induced inflammation. Various forms of therapy induce death (necrosis) of malignant cells resulting in the release of necrotic products and damage-associated molecular patterns (DAMPs) that activate cytokine-producing inflammatory cells. These cytokines activate pro-survival genes in residual cancer cells, rendering them resistant to subsequent rounds of therapy. However, in some cases, therapy-induced inflammation augments the presentation of tumor antigens and stimulates an anti-tumor immune response that improves the therapeutic outcome.

Table 1

Roles of different subtypes of immune and inflammatory cells in anti-tumor immunity and tumor-promoting inflammation

Cell types	Anti-tumor	Tumor-promoting
Macrophages, dendritic cells, myeloid-derived suppressor cells	Antigen presentation Production of cytokines (IL-12 and type I IFN)	Immunosuppression Production of cytokines, chemokines, proteases. growth factors, and angiogenic factors
Mast cells		Production of cytokines
B cells	Production of tumor specific antibodies?	Production of cytokines Activation of mast cells Immunosuppression
$CD8^+$ T cells	Direct lysis of cancer cells Production of cytotoxic cytokines	Production of cytokines?
$CD4^+$ Th2 cells		Education of macrophages Production of cytokines B cell activation
$CD4^+$ Th1 cells	Help to cytotoxic T lymphocytes (CTLs) in tumor rejection	Production of cytokines
	Production of cytokines (IFN□)	
$CD4^+$ Th17 cells	Activation of CTLs	Production of cytokines
$CD4^+$ Treg cells	Suppression of inflammation (cytokines and other suppressive mechanisms)	Immunosuppression Production of cytokines
Natural Killer cells	Direct cytotoxicity toward cancer cells Production of cytotoxic cytokines	
Natural Killer T cells	Direct cytotoxicity toward cancer cells Production of cytotoxic cytokines	
Neutrophils	Direct cytotoxicity Regulation of CTL responses	Production of cytokines, proteases, and ROS

Exhibit 66

Inflammation and cancer: advances and new agents

Shanthini M. Crusz and Frances R. Balkwill

Abstract | Tumour-promoting inflammation is considered one of the enabling characteristics of cancer development. Chronic inflammatory disease increases the risk of some cancers, and strong epidemiological evidence exists that NSAIDs, particularly aspirin, are powerful chemopreventive agents. Tumour microenvironments contain many different inflammatory cells and mediators; targeting these factors in genetic, transplantable and inducible murine models of cancer substantially reduces the development, growth and spread of disease. Thus, this complex network of inflammation offers targets for prevention and treatment of malignant disease. Much potential exists in this area for novel cancer prevention and treatment strategies, although clinical research to support targeting of cancer-related inflammation and innate immunity in patients with advanced-stage cancer remains in its infancy. Following the initial successes of immunotherapies that modulate the adaptive immune system, we assert that inflammation and innate immunity are important targets in patients with cancer on the basis of extensive preclinical and epidemiological data. The adaptive immune response is heavily dependent on innate immunity, therefore, inhibiting some of the tumour-promoting immunosuppressive actions of the innate immune system might enhance the potential of immunotherapies that activate a nascent antitumour response.

Crusz, S. M. & Balkwill, F. R. *Nat. Rev. Clin. Oncol.* **12**, 584–596 (2015); published online 30 June 2015; doi:10.1038/nrclinonc.2015.105

Introduction

Innate inflammation is usually self-limiting, with active resolution through apoptosis and clearance of debris and immune cells. Chronic, dysregulated, persistent, and unresolved inflammation is associated with an increased risk of malignant disease. Almost 20% of human cancers are related to chronic inflammation caused by infections, exposure to irritants or autoimmune disease.[1] Common causes of chronic inflammation associated with cancer development include *Helicobacter pylori* infections in gastric mucosa-associated lymphoid tissue lymphoma, Hepatitis B or C infections in hepatocellular carcinoma (HCC) and inflammatory bowel disease in colorectal cancer (CRC).

Cells and mediators of the innate immune system are detected in most, if not all cancers, regardless of whether or not extrinsic inflammation is implicated in their development. One of the reasons for this high prevalence of mediators of the innate immune system is that oncogenic changes in cells lead to induction of inflammatory pathways in pre-malignant and malignant cells (Figure 1); thus, not only can inflammation cause cancer, but cancer also causes inflammation.

Inflammatory cells and mediators (including cytokines, chemokines and prostaglandins) in the tumour microenvironment coordinate a milieu of proinflammatory responses, which can act in an autocrine and/or paracrine manner on both malignant and nonmalignant cells.[2] Immune-cell infiltration of tumours can have a dual role: either leading an antitumour response, or active promotion of tumorigenesis and inhibition of a protective immune response.[3] Thus, the composition of the tumour inflammatory microenvironment has a pivotal influence on disease outcome.

Our understanding of cancer-related inflammation continues to increase, and we are beginning to translate this knowledge to the development of therapeutic targets of pro-tumorigenic immunity, leading to new approaches to cancer prevention and treatment (Table 1).[4] This Review will focus on the evidence for the use of established anti-inflammatory agents in the prevention and treatment of human cancer, explores early phase trials that target inflammatory cells and inflammatory mediators, and discusses the future prospects for translating our knowledge of cancer-related inflammation into clinical practice.

Anti-inflammatory agents

Cyclooxygenase (COX)-2 enzymes have an important function in driving tumorigenesis through the production of prostaglandins, which in turn act directly on cancer cells to inhibit apoptosis and enhance cell migration, and also act on stromal tissue to promote neoangiogenesis.[5] COX-2 levels are raised in several tumour types, such as those of the breast,[6] prostate,[7] pancreas,[8] skin,[9] lungs,[10] bladder[11] and head and neck.[12] Accordingly, established agents that target COX-2 in the treatment of other diseases have been investigated for effectiveness as treatments of cancer.

Barts Cancer Institute, Queen Mary University of London, John Vane Science Centre, Charterhouse Square, London EC1M 6BQ, UK (S.M.C., F.R.B.).

Correspondence to: F.R.B. f.balkwill@qmul.ac.uk

Competing interests
The authors declare no competing interests.

Key points

- Inflammation and innate immunity have a vital and complex role in driving tumorigenesis
- Strong epidemiological and preclinical data support an anti-inflammatory approach to prevention and treatment of cancer
- Several therapeutic agents targeting inflammatory cytokines, transcription factors and immune cells are being developed and tested in the clinical setting
- Current successes of treatments targeting adaptive immunity indicate a great need to further our clinical understanding of the inflammatory and innate immune system to identify further targets for cancer treatment
- Combining treatments that target the adaptive and innate immune systems in the tumour microenvironment might be advantageous

NSAIDs are a family of compounds that primarily inhibit the activity of COX enzymes and thereby reduce the synthesis of prostaglandins. Following initial observations that the use of NSAIDs leads to regression of rectal polyp formation in patients with Gardener syndrome,[13] the use of NSAIDs in preventing tumorigenesis has been considered.

Extensive epidemiological data suggest a beneficial effect of NSAIDs in the prevention of cancer. In 2012, Peter Rothwell and collaborators[14–16] conducted the largest pooled analysis of observational and randomized trials that explored the effects of daily aspirin use on cancer development and dissemination to date. These three seminal papers[14–16] strongly supported the favourable effects of aspirin in the prevention and development of cancer in large patient cohorts. Through systematic review of case control and cohort studies, Rothwell and co-workers[14] reported that regular use of aspirin reduced the risk of CRC, in agreement with published data from previous randomized trials. Reductions in the risk of oesophageal, gastric, biliary and breast cancer were also reported.[14] In these observational studies, the regular use of aspirin was associated with a reduced proportion of cancers with distant metastasis, but was not associated with a reduction in regional spread, which was also in keeping with findings of previous randomized trials.[14] In addition, Rothwell and colleagues analysed deaths owing to cancer in randomized trials originally designed to test the use of aspirin for the prevention of vascular events. A significant reduction in overall cancer-associated mortality was seen in patients who received aspirin compared with those who did not (odds ratio, OR 0.85, 95% CI 0.76–0.96; $P=0.008$);[15] incidence of cancer was reduced after 3 years of treatment, and cancer-related mortality was reduced only after 5 years.[15] The authors found that aspirin use again correlated with a reduced risk of metastatic disease in these randomized trials, particularly in patients with adenocarcinomas, who had a reduced risk of metastasis at the time of diagnosis (HR 0.69; $P=0.02$) and a reduced risk of subsequent development of metastases following the detection of local disease at presentation (HR 0.45; $P=0.0009$).[16] These epidemiological studies strongly support the use of aspirin in treating cancers and preventing metastatic dissemination, particularly in patients with adenocarcinomas.

The adverse-effect profile of aspirin and NSAIDs can be substantial, including an increased risk of major bleeding. Thus, the benefits of taking these drugs as a prophylactic measure for prevention of cancer must be assessed by risk versus benefit analyses. Cuzick *et al.*[17] have addressed this issue through a systematic review published in January 2015, showing an overall net benefit of 5 years of daily aspirin use in persons aged between 50–65 years of age, and an increased risk of adverse effects with advancing age.[17]

The action by which aspirin and other NSAIDS prevent cancer has yet to be fully determined; on a cellular level, evidence suggests that platelets drive tumour growth and metastasis. Platelet activation is hypothesized to result in the formation of aggregates that surround tumour cells and provide protection from the immune system, as well as promotion of tumour-cell adhesion to the endothelium, resulting in tumour-cell arrest and extravasation.[18] A proinflammatory environment within a tumour will also result in platelet-mediated aberrant expression of COX-2. Thus, the antiplatelet effects of aspirin and COX-2 inhibition of NSAIDs have been proposed as the most plausible mechanisms for their antitumour effects. Research using mouse models of CRC confirms that COX-2 levels are substantially higher in malignant tissue compared with the normal mucosa,[19] but the effects of NSAIDs on other tumours with high COX-1 and COX-2 expression have been equivocal or modest at best,[20,21] suggesting other pathways and mechanisms of action are also involved.

Furthermore, the findings of several studies suggest improved long-term survival is associated with the use of aspirin after surgery in patients with CRC with a mutated PI3Kα phenotype,[22] and for post-diagnosis patients with breast cancer.[23] Hence, having shown a clear epidemiological link between the use of NSAIDs and cancer risk, clinicians are now exploring the role of this class of drugs in treatment and prevention of cancer. Prospective observational data support this strategy,[24] showing that the use of aspirin and COX-2 inhibitors in conjunction with adjuvant chemotherapy in patients with stage III colon cancer improves recurrence-free survival (HR 0.51; 95% CI 0.28–0.95).[25] Accordingly, several phase III trials evaluating aspirin and NSAIDs in the perioperative setting in patients with CRC are currently ongoing,[26,27] and the 'Add-Aspirin' trial,[28] which will investigate aspirin use in several early stage cancers, is soon to open. At a molecular level, further clarification of the mechanism(s) underlying the effects of NSAIDs on cancer risk will enable the discovery of alternative novel therapeutic approaches to target this seemingly relevant pathway.

Similarly to aspirin, other agents with conventional anti-inflammatory actions are being repurposed for use in the treatment of cancer. One such class of agents are the 3-hydroxy-3-methy-glutaryl-CoA (HMG-CoA) reductase inhibitors (statins), which have a wide spectrum of activity as anticancer agents, including antiangiogenic and anti-inflammatory actions, as demonstrated in preclinical studies.[29] The effects of these agents are variable in other cancer types and an established association exists between statin use and a reduced risk of advanced-stage and/or

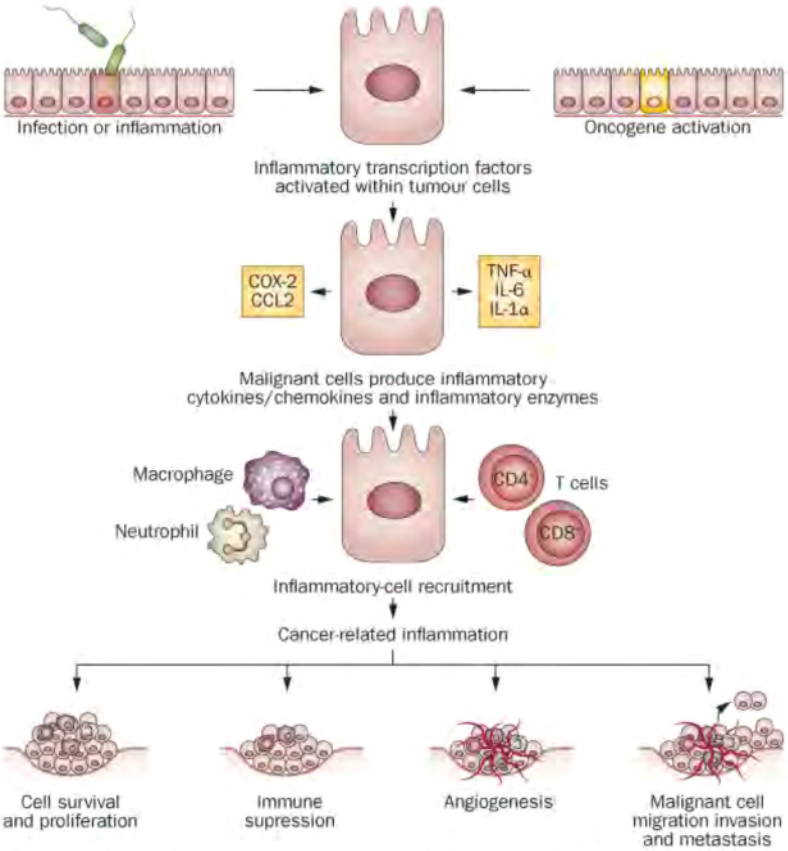

Figure 1 | The molecular basis of cancer-related inflammation. Inflammation or oncogene activation results in the expression of vital proinflammatory transcription factors within tumour cells (such as NF-κB, STAT3 or HIF1α). These activated transcription factors mediate the expression of key cytokines and chemokines (including TNFα and IL-6) as well as inflammatory enzymes (such as COX-2), forming a rich and complex network of inflammatory responses within the tumour microenvironment. Host leukocytes, including macrophages, dendritic cells, mast cells and T cells are recruited by chemokines, and function within the tumour stroma to mediate the immune response. Autocrine and paracrine effects of cytokine release within the tumour microenvironment might further sustain these cells. Inflammatory enzymes catalyse key steps in prostaglandin synthesis, which further regulate a number of physiological processes involved in cancer-related immunity and inflammation, thus driving the process of tumorigenesis. Abbreviations: COX-2, cyclo-oxygenase-2; HIF1α, hypoxia-inducible factor 1α; IL-6, interleukin-6; NF-κB, nuclear factor-κB; STAT3, signal transducer and activator of transcription 3; TNFα, tumour necrosis factor-α. Permission obtained from Nature Publishing Group © Mantovani, A. *et al.* Cancer-related inflammation. *Nature* **454**, 436–444 (2008).[2]

aggressive prostate cancer.[30] However, a definitive benefit of statin use has not been demonstrated in a randomized controlled trial setting to date—several trials in patients with different tumour types have been designed to answer this question and are currently ongoing.[31,32]

Cachexia is strongly associated with inflammation in patients with cancer; cachexia is a complex process, in which proinflammatory cytokines are likely to be implicated.[33] This cancer-associated symptom has a marked effect on patients' quality of life (QoL), and is associated with inferior outcomes compared with patients without cachexia.[34] Anti-inflammatory steroids are commonly used as supportive medications and as appetite stimulants in patients with cancer-related cachexia. The pre-administration of steroids has been shown to enhance the effectiveness and reduce the toxicity of anticancer therapies in a phase I/II clinical trial;[35] however, a specific antitumour effect needs to be confirmed in a phase III clinical trial setting.

Targeting cytokines and chemokines

Cytokines and chemokines are molecular messengers that enable cells of the immune system to coordinate a self-limiting immune response. In patients with normal immune-system function, a complex cytokine network forms to mediate an effective response. Cytokines and chemokines are released by both tumour and host cells (such as stromal, endothelial and/or immune cells) within the tumour microenvironment. These inflammatory mediators have direct effects on tumour-cell and myeloid-cell function, and contribute to several hallmarks of cancer,[36] such as stimulation of the epithelial-to-mesenchymal transition (EMT) and augmentation of metastasis.[37,38]

Furthermore, selective inhibition of the actions of certain cytokines might affect the extensive tumour cytokine network (Box 1). Dysregulated cytokine production might also contribute to cancer-related cachexia;[39] therefore, targeted modification of cytokine signalling has the potential to achieve systemic as well as tumour-specific therapeutic effects.

Established biological agents targeting TNF-α

Tumour necrosis factor-α (TNF-α) is a key multifunctional cytokine with pleiotropic actions in the regulation of immune responses associated with inflammation. TNF-α functions through binding to two receptors (TNF receptor [TNFR]1 and TNFR2) resulting in regulation of cytokines, proteases and growth-factor production.[40]

Biological agents that target the actions of TNF-α include infliximab, etanercept and adalimumab. These agents are commonly used to treat several inflammatory conditions, including rheumatoid arthritis and Crohn's disease.[41,42] Infliximab is a chimeric mouse–human monoclonal antibody that binds to soluble and membrane bound TNF-α and prevents it from binding to its receptors,[43] etanercept is a recombinant TNFR2 fusion protein that binds to soluble TNF-α,[44] and adalimumab is a fully humanized monoclonal antibody that also binds and antagonizes TNF-α.[45]

Both the tumour and stromal cells of solid tumours secrete TNF-α. This cytokine was originally named owing to its antitumour properties, having been found to induce haemorrhagic necrosis of tumours.[46] Since this discovery, however, TNF-α has been shown to have a paradoxical effect on cancers by both promoting and inhibiting tumour growth.[47] Chronically produced TNF-α enhances tumour development and dissemination, with increased serum levels of this cytokine detected in patients with several cancers.[48,49] TNF-α is also thought to have a role in fatigue and cachexia associated with malignancy.[50] As a major cytokine in the tumour microenvironment, which is capable of regulating other cytokines and chemokines, TNF-α is able to influence several of the hallmarks of cancer, including stimulation of tumour-cell

Table 1 | Anti-inflammatory agents tested in clinical trials in patients with cancer*

Agent	Inflammatory target	Class of agent	Study type/tumour type
Non-specific agents			
NSAIDs (including aspirin)	COX-2?	To be determined: anti-platelets or COX-2	Epidemiological evidence[14–16] Chemoprevention and/or perioperative trials (pending)[26–28]
Cytokines and chemokines			
Infliximab	TNFα	Chimeric TNFα-specific antibody	Phase I/II; RCC[51,52] Phase I/II; pancreatic cancer[53]
Etanercept	TNFα	Human TNFR2–Fc fusion protein	Phase II; ovarian[58] and breast cancer[55]
Siltuximab	IL-6	Chimeric anti-IL-6 antibody	Phase I; Castleman's disease (approved for use)[76] Phase I/II; MM,[141] prostate cancer,[79,81,82] and RCC[83] Phase II; ovarian cancer[73]
Tocilizumab	IL-6	Human IL-6R-specific antibody	Phase I/II; ovarian cancer (pending)[192]
Carlumab	CCL2	Human anti-CCL2 antibody	Phase I[87] Phase II; prostate cancer[88]
MABp1	IL-1α	True human anti-IL-1α antibody	Phase I[93] Phase III; colorectal cancer (pending)[193]
Reparixin	CXCR1/2	Small-molecule inhibitor	Phase I; breast cancer (pending)[194]
Plerixafor	CXCR4	Small-molecule inhibitor	Phase I; pancreas, ovarian and colorectal cancer (pending)[195]
Inflammatory transcription factors			
Ruxolitinib	JAK1/2	Small-molecule inhibitor	Phase III; myelofibrosis[129,130]
Pacritinib	JAK2	Small-molecule inhibitor	Phase I; lymphoma[131] Phase III; myelofibrosis (pending)[196] Phase II; colorectal cancer (pending)[197]
Bortezomib	NF-κB	Proteosome inhibitor	Phase III; MM[137,138] Phase II; mantle-cell lymphoma[139]
Inflammatory cells			
Trabectedin	TAMS	Selective monocyte/macrophage cytotoxicity	Phase III; sarcoma (approved)[198] Phase I; solid tumour (pending)[199]
RG7155/IMC-CS4/PLX3397/AMG 820	CSF-1R	Human CSF-1R specific antibodies/TKIs	RG7155: phase I; tenosynovial giant-cell tumour[165] IMC-CS4/AMG 820/PLX3397: solid malignancies (pending)[200–202]
CP-870,893	CD40	Human CD40 agonist antibody	Phase I; pancreatic cancer[178]
Tasquinimod	MDSCs	Quinolone-3-carboxamide	Phase I/II; prostate cancer[180,181] Phase I/II/III; prostate cancer (pending)[203]

*This is not a comprehensive table but lists agents and examples of trials referenced in this Review. Abbreviations: CCL2, CC chemokine ligand 2; COX-2, cyclooxygenase-2; CSF-1R, macrophage colony-stimulating factor receptor-1; CXCR(1/2/4), CXC chemokine receptor (1/2/4); IL-1α, interleukin-1α; IL-6, interleukin-6; IL-6R, interleukin-6 receptor; MDSCs, myeloid-derived suppressor cells; MM, multiple myeloma; NF-κB, nuclear factor-κB; NSAIDs, non-steroidal anti-inflammatory drugs; RCC, renal-cell carcinoma; TAMs, tumour-associated macrophages; TKIs, tyrosine-kinase inhibitors; TNFα, tumour necrosis factor-α; TNFR2–Fc, tumour necrosis factor receptor 2–IgG1 Fc fragment fusion protein.

growth, survival, invasion, metastasis and inflammatory-cell trafficking and neoangiogenesis.[40] Thus, owing to the successful use of agents that affect TNF-α signalling in inflammatory conditions, interest in using these agents to target TNF-α for the treatment of various malignancies has been explored.

Infliximab monotherapy has potential therapeutic benefit for the treatment of patients with renal-cell carcinoma (RCC), with 61% of patients having clinical benefit—as defined by Response Evaluation Criteria In Solid Tumours (RECIST) partial response (PR) or stable disease (SD) >3 months—at a dose of 10 mg/kg in the phase II setting.[51] This finding led to the testing of this agent in combination with the tyrosine-kinase inhibitor (TKI) sorafenib; however, unacceptable toxicities were observed with no improvement in efficacy compared to sorafenib monotherapy.[52] In patients with pancreatic cancer, combination therapy with gemcitabine resulted in no excess toxicity, but again, had no benefit over monotherapy.[53] In patients with breast cancer, in whom elevated serum TNF-α concentration is associated with more-aggressive tumour biology,[54] etanercept was tested in the phase II setting for the treatment of progressive metastatic disease.[55] Etanercept was well tolerated, and showed signs of biological activity through significant declines in CCL2 and nonsignificant declines in IL-6 levels in patients treated with this agent; however, limited anticancer activity was noted in this small, heavily pre-treated population of patients—none of seven patients had RECIST-defined complete response (CR) or PR, one of seven had SD and six of seven had progressive disease).[55] In patients with advanced-stage ovarian cancer, in whom TNF-α expression is confined to malignant tissue and correlates with tumour grade,[56,57]

Box 1 | Targeting cytokines and chemokines

Cytokines and chemokines prove attractive targets of anticancer therapy for many reasons:

- Several cytokines are overexpressed in malignant tissue by both host and malignant cells, and are known to have both prognostic as well as predictive implications in patients with certain tumour types
- Through targeting cytokines and chemokines, therapeutic modulation of not only tumour cells, but also stromal tissue becomes possible, which can take place in both a paracrine and autocrine manner; the advantage of stromal targeting is that these cells are genetically stable compared with cells of a cancerous phenotype, thus, hypothetically decreasing the risk of resistance
- The cytokine network within the tumour microenvironment is complex. One cytokine can influence several cell functions; therefore, this poses an attractive therapeutic target because one agent might have multiple actions

etanercept treatment resulted in prolonged disease stabilization in six out of 30 patients, each having a consistent decrease in IL-6 and CCL2 over time, confirming meaningful biological activity of etanercept.[58]

Interestingly, in the context of treating inflammatory conditions, use of TNF blockers has been suggested to cause lymphoproliferative malignancies and skin cancers. This effect has been most-extensively investigated in patients with rheumatoid arthritis, and many studies have attempted to clarify the evidential basis of this suggested association. Observational studies detected no association between treatment with anti-TNF agents and malignancy over time.[59–62] One meta-analysis of 21 prospective observational studies of the effects of anti-TNF agents in patients with rheumatoid arthritis found no increase in risk of lymphoma, although an increase in the risk of skin cancers, including melanoma, was reported.[63] Further meta-analyses of randomized clinical trial data detected no significant increase in any type of cancer in patients with rheumatoid arthritis who received anti-TNF biological agents.[64–66] However, to date, there is also no evidence of a decreased risk of cancer development and dissemination in this patient group.

Interleukin-6

IL-6 is another major tumour-promoting cytokine produced by both malignant and host cells within the tumour microenvironment.[67] IL-6 is also a downstream product of oncogenic mutations in genes such as *RAS* family members and *TP53*.[68,69] IL-6 has both local and systemic tumorigenic actions in experimental models of cancer, and in patients with cancers, which are typically mediated by its major downstream signal transducer, signal transducer and activator of transcription 3 (STAT3).[67] These actions include stimulation of malignant-cell growth and survival, promotion of invasion and metastasis, modulation of tumour-promoting T-cell subtypes, involvement in autocrine tumour cell cytokine networks and regulation of the myeloid cell infiltrate. Excess IL-6 production in patients with cancer not only drives tumorigenesis, but also induces acute phase reactants and elevates platelet counts (paraneoplastic thrombocytosis).[70] Raised circulating IL-6 levels indicate a poor prognosis of patients with several types of cancer, including ovarian cancer,[71,72] multiple myeloma[73] and HCC.[74]

Siltuximab, a chimeric mouse–human IgG_1 monoclonal antibody, binds to IL-6 with high affinity and specificity.[75] This monoclonal antibody is currently licensed for treatment of Castleman's disease, an atypical lymphoproliferative disorder, which is characterized by overproduction of IL-6 from affected lymph nodes, resulting in systemic manifestations. In a phase I trial that investigated siltuximab as a treatment of Castleman's disease, the clinical benefit response (CBR; a composite outcome measure incorporating haemoglobin level, fatigue, anorexia, fever/night sweats, weight and largest lymph node size) was 86% in patients with Castleman's disease, with a third of patients having a radiological response.[76] This same study demonstrated clinical effectiveness of siltuximab in patients with multiple myeloma with 15% ($n = 2$) of patients having a CR to treatment.[76] However, in a further trial investigating the effectiveness of this combination therapy, no clinical benefit was observed with siltuximab plus chemotherapy in transplant-ineligible patients with multiple myeloma receiving first-line therapy, or patients who had relapsed disease.[77]

A single arm, phase II trial of siltuximab in patients with chemoresistant ovarian cancer demonstrated the therapeutic potential of targeting the IL-6-signalling pathway: although no CR or PR by RECIST criteria were achieved, one patient had an overall PR by combined RECIST/cancer antigen (CA)-125 criteria (normalization of serum CA-125 levels lasting 12 weeks) and eight patients had SD, which persisted for 6 months or more in four patients.[78] In patients with SD for >6 months, the IL-6-regulated plasma concentrations of the chemokines CCL2, CXCL12 and VEGF declined substantially,[78] demonstrating biological activity of this agent through downregulation of other cytokines involved in various aspects of tumorigenesis. When used as a monotherapy in patients with prostate cancer, siltuximab had minimal clinical activity in an open-label phase II trial for patients with castration-resistant disease who had previously received docetaxel.[79] Evidence exists that siltuximab can sensitize cancer cells to cytotoxic treatments in patients with prostate cancer;[80] thus, this agent has been tested in combination with other chemotherapies. Specifically, a phase I combination study with docetaxel has shown this combination regimen to be safe, and 23 out of 37 patients who received this treatment had a ≥50% serum PSA response with two of 17 having confirmed radiological PRs.[81] A phase II study of combination siltuximab with or without mitoxantrone and prednisolone, however, detected inferior progression-free survival (PFS) compared with mitoxantrone and prednisolone alone. Of note, enrolment in this study was prematurely terminated owing to an imbalance in baseline patient characteristics (favouring the mitoxantrone and prednisolone arm), and the trial never reached its primary efficacy end point and results are thus considered inconclusive.[82] In the setting of RCC, a phase I/II study of siltuximab monotherapy detected stabilized disease in >50% of patients with progressive metastatic disease, and demonstrated a favourable toxicity profile, but this agent has currently not been investigated further in this disease setting.[83]

Tocilizumab (an anti-IL-6 receptor antibody) is licensed for the treatment of patients with rheumatoid arthritis, systemic juvenile idiopathic arthritis and Castleman's disease. A case report published in 2013 has indicated the potential of this agent for the management of patients with cancer, as it was shown to be effective in treating cachexia in a patient with large-cell lung carcinoma.[84] Certainly, the biological data suggest that the therapeutic rationale in this context is justified, and thus outcomes of further clinical trials, especially of IL-6 receptor antagonists in rational combinations with other targeted therapies, will be of interest. In this respect, we have shown upregulation of EGFR pathways in ovarian cancer cells *in vitro* and *in vivo* following treatment with neutralizing antibodies to IL-6.[78] By blocking the EGFR pathway with gefitinib, an inhibitor of the EGFR tyrosine-kinase domain, in combination with neutralizing antibodies to IL-6, malignant-cell growth was abolished in 2D and 3D cultures, and this treatment also resulted in reduced tumour weight in *in vivo* models.[85] This finding offers a preclinical rationale for combination therapy in patients with advanced-stage ovarian cancer, and also highlights the presence of crosstalk between inflammatory and other tumorigenic signalling pathways in malignant cells.

CCL2

CCL2, an inflammatory chemokine, is highly expressed in both the tumour-cell and stromal-cell populations of many cancers. This chemokine is hypothesized to directly stimulate tumour-cell proliferation, survival and migration, influence angiogenesis, and act as a chemotactic factor to tumour cells and inflammatory monocytes.[86] The results of a phase I study published in 2013 have shown that blocking the function of CCL2 with carlumab, a human anti-CCL2 IgG_1 antibody, was well tolerated and resulted in some serological and radiological responses in patients with advanced-stage cancer.[87] This agent has been tested in the phase II setting in patients with castration-resistant prostate cancer; however, carlumab did not block the CCL2–CCR2 axis or have antitumour activity as a single agent.[88]

Intriguingly, data published in the past 3 years raise the potential need for caution regarding the use of anti-CCL2 treatment. Findings in mouse models suggest that discontinuing anti-CCL2 treatment results in increased metastasis, leading to accelerated death, owing to a rebound in circulating monocyte levels.[82] Speculations are that this increased risk of mortality could be a result of one of two possible effects: either the drug prevents mobilization of monocytes causing levels to accumulate in the bone marrow, with a heightened release of these cells into the circulation when treatment is discontinued; or, cessation of treatment causes increased CCL2 levels at metastatic sites after therapy.[89] Certainly, free CCL2 levels surged in patients treated with carlumab as early as 1 week post-infusion.[88] These complexities of targeting immune cells and immunity within cancer treatment have been highlighted in the context of anti-CCL2 therapy, although the same issues could apply to other immune-modulating therapies.

Interleukin-1α

The production of IL-1α drives early phase inflammation, and in cancers this cytokine is associated with dedifferentiated and more-aggressive disease.[90–92] This cytokine functions through activation of the vascular endothelium, causing infiltration of tumorigenic inflammatory cells. Consequently IL-1α as a tumorigenic cytokine is a promising therapeutic target. The therapeutic agent developed to target this factor is a unique first-in-class antibody: a 'true human' IgG_1 natural antibody, cloned from an affinity-matured *in vivo* human immune response, with no sequence modifications that alter binding affinity.[93] IL-1α is expressed on the surface of platelets, monocytes and tumour cells. In the phase I setting, this drug has shown promising results through measurement of tumour response, QoL-index scoring and lean body mass; out of 42 patients available for RECIST analysis, eight had SD for at least 3 months, and one had a PR.[93] Lean body mass, as measured by dual energy X-ray absorptiometry (DEXA), was shown to be increased in 70% of patients and treatment was associated with improvement in fatigue, pain and appetite loss.[93] A decrease in IL-6 from baseline to week 8 of treatment (although not significant) was similar to the increase in lean body mass,[93] suggesting the drug has biological activity in targeting cytokine-related cachexia in patients with cancer. Thus, this drug is being taken forward for further analysis and testing, initially in patients with colorectal cancer.

Interleukin-8

IL-8, also known as CXCL8, has multiple biological functions,[94] including acting as a chemotactic factor for T cells, neutrophils and basophils.[95–97] As a chemokine, this agent has both tumorigenic and antitumour effects. It promotes tumorigenesis by inducing migration of tumour cells,[98] promoting angiogenesis,[99,100] and enhancing metastasis in patients with melanoma.[101] A study by Sanmamed *et al.*[102] published in 2014 has implicated this cytokine as a potential biomarker of malignancy, with serum levels correlating with tumour burden and prognosis in several tumour types, as well as with the extent of clinical responses to novel therapies such as BRAF inhibitors and immunomodulatory antibodies, in patients with melanoma. Conversely, IL-8 has been suggested to have an antitumour effect in patients with ovarian cancer by causing neutrophil infiltration, which results in decreased tumour growth, and has been proposed to have a role in the treatment effect of paclitaxel observed in ovarian cancer cell lines.[103,104] Nevertheless, studies to date have aimed to inhibit the actions of IL-8, and a large proportion of studies have focused on the treatment of inflammatory conditions. In cancer treatment, targeting IL-8 using neutralizing antibodies has been shown to have antiangiogenic effects, resulting in reduced tumour burden in mouse models.[104,105] Despite promising preclinical studies in mouse models of melanoma, which demonstrated reduced tumour growth and angiogenesis in nude mice xenografts treated with anti-IL-8 antibodies,[106] clinical application of direct

anti-IL-8 therapies in patients with cancer has not been widely investigated. Inhibition of ligand binding at the extracellular domain of CXCR1 and CXCR2, the cellular receptors for IL-8, is an alternative option to reduce the effects of this chemokine. This approach has been tested *in vitro*, through treatment of small-cell lung cancer cell lines with anti-CXCR1 antibodies, resulting in reduced cell proliferation.[107] *In vivo*, treatment with anti-CXCR2 antibodies resulted in reduced tumour volume and microvessel density in orthotopic pancreatic mouse models.[108] Clinical trials investigating reparixin, a small-molecule inhibitor of CXCR1/2, both as a monotherapy and in combination with chemotherapy, are currently ongoing.[109,110] The hypothesized mechanism of action of reparixin involves targeting of breast cancer stem cells, in light of evidence of reduced tumour growth and metastasis in breast cancer xenograft models.[111]

CXCR4

The CXCL12–CXCR4 pathway is emerging as a new potential therapeutic target for metastatic cancer. CXCL12 signals via the cell-surface receptor CXCR4 and was originally identified in lymphocytes trafficking to the bone marrow. CXCR4 is highly expressed on malignant cells in a large number of tumour types, including those of the breast,[112] ovaries,[113] prostate,[114] kidney,[115] skin (melanoma)[116] and brain[117] and is understood to have a role in tumour dissemination and metastasis; CXCL12 protein levels are highest in organs that are known to be common sites of metastases, such as the liver, bone marrow and lungs.[112] Tumour-associated fibroblasts are also likely to contribute to CXCR4/CXCL12 signalling within the tumour microenvironment, and they function directly through promotion of tumour-cell growth, matrix metalloproteinase (MMP)-mediated tissue remodelling, neoangiogenesis and EMT in primary tumour cells.[118–120]

Agents targeting CXCR4, such as plerixafor, were originally developed for use in the treatment of HIV (as CXCR4 is a co-receptor for this virus) and are licensed for use in malignant conditions to mobilize stem cells for harvesting before bone-marrow transplantation. In the context of solid malignancies, preclinical data in several tumour types have suggested that blocking this pathway leads to decreased metastasis and could be used to improve treatment effectiveness when given in combination with other anticancer treatments.[121–126] Thus, an early phase trial[127] designed to test the clinical effects of targeting this pathway will be of particular interest.

Targeting transcription factors

JAK/STAT inhibitors

Janus kinases (JAKs) are a family of four intracellular nonreceptor tyrosine kinases (JAK1, JAK2, JAK3 and TYK2) that act as primary signal transducers from cell-surface receptors activated by cytokines and growth factors. These kinases in turn phosphorylate STAT proteins on tyrosine residues, promoting their functions as transcription factors in the expression of several genes involved in cell survival. These pathways are dysregulated in many cancers.[128] Ruxolitinib, a selective JAK1/2 inhibitor, was proven to be clinically effective in two randomized phase III trials through marked improvements in splenomegaly and disease-related symptoms compared with either placebo,[129] or the best available therapy,[130] and has thus gained approval for clinical use in patients with myelofibrosis. Pacritinib, a potent inhibitor of JAK2, has been tested in the phase I setting for the treatment of relapsed lymphoma[131] and is currently being trialled in the phase III setting for treatment of myelofibrosis.[132]

Preclinical studies have linked the JAK–STAT pathway to therapy resistance in patients with lung cancer, and the activity of this pathway is regulated by IL-6 in lung cancer cell lines with activating *EGFR* mutations.[133] The results of *in vitro* investigations suggest that in *EGFR*-mutated lung cancer cell lines with constitutive IL-6-mediated STAT3 activity, siltuximab in conjunction with erlotinib (an EGFR inhibitor) suppresses STAT3 transcriptional activity and reduces cell proliferation.[133] *In vivo*, this combination treatment resulted in a substantial reduction in tumour size compared with the control or monotherapy groups.[134] Thus, inhibition of cytokine signalling in conjunction with JAK–STAT pathway inhibitors might prove effective as a treatment for patients with *EGFR*-mutant lung cancers and has been proposed for an early phase trial.

Nuclear factor-κB

The nuclear factor-κB (NF-κB) transcription factor family has a pivotal role in orchestrating inflammation and is a key participant in innate and adaptive immune responses. In most cancers, NF-κB is constitutively active and has a role in oncogenic transformation through aberrant activation; through activation of antiapoptotic and cell-cycle progression genes, NF-κB drives chronic inflammation, thus promoting tumour development.[135] Inhibition of the NF-κB pathway could, hypothetically, be an effective treatment approach for many cancers, although in reality, targeting this protein has proven difficult owing to its complex biological function, which has resulted in substantial toxicities with some treatments.[136] One successful NF-κB-inhibiting agent, however, is now used in the treatment of patients with multiple myeloma and relapsed mantle-cell lymphoma: the proteasome inhibitor, bortezomib. Patients with multiple myeloma who are ineligible for autologous stem-cell transplantation can be treated with bortezomib in combination with melphalan and prednisone (VMP regimen) based on findings of the VISTA study, which detected an impressive improvement in the CR rate (30% versus 4%; $P<0.001$) and overall survival (median 56.4 months versus 43.1 months; $P<0.001$) with the novel treatment arm compared with melphalan plus prednisone alone.[137,138] Treatment with bortezomib also resulted in an improved overall response rate (ORR) and prolonged PFS in a heavily pretreated population of patients with chemotherapy-refractory mantle-cell lymphoma in a phase II trial,[139] but remains to be tested in the randomized controlled trial setting for earlier-stage disease.

Owing to the links between IL-6 and early development of multiple myeloma,[140] the addition of siltuximab to bortezomib has been evaluated, but has not shown significant benefits relative to bortezomib alone in the relapsed disease setting,[141] or as a first-line treatment in combination with the VMP regimen in patients who are transplant ineligible.[142]

Targeting inflammatory cells

As previously described, immune cells within the tumour microenvironment substantially influence tumour responses. When a successful immune reaction is mounted, tumour-associated antigens activate the adaptive immune response, enabling direct cytotoxic effects on cancer cells to be achieved, with the development of long-term 'memory' and thus lasting effects. Certainly, clinical findings support this hypothesis, with more-favourable outcomes in patients with tumours harbouring activated T-lymphocyte infiltrates.[143,144] These findings suggest that trying to promote an adaptive-immune profile within the tumour microenvironment would be beneficial. However, many cancers have developed mechanisms to evade immune recognition and actively suppress immunity. The successes of therapy with immune-checkpoint inhibitors, such as anti-cytotoxic-T-lymphocyte protein-4 (CTLA-4) and anti-programmed cell death 1 ligand 1 (PD-L1) antibodies, indicate exciting therapeutic possibilities in trying to counteract tumour evasion of the host immune response.[145–148] Nonetheless, pharmacological immune-checkpoint blockade has not been universally successful in all tumour types, and other strategies, especially those targeting the innate immune system, are being further explored to modulate inflammation-related cancer. Suppression of cancer-related inflammation might enhance the effectiveness of immune-checkpoint blockade.

Tumour-associated myeloid-derived cells are thought to have an important role in tumorigenesis. Clinical evidence links the density of macrophages in the tumour microenvironment with worse prognosis; in one analysis over 80% of studies indicated that higher macrophage density was associated with inferior patient prognosis.[149] Not all macrophages function to promote cancer progression, however, and macrophage phenotype is hypothesized to strongly dictate their function within the tumour microenvironment: a simplistic view exists that 'classically activated macrophages' (M1 subtype) have an antitumour effect and 'alternatively activated' cells (M2 subtype) have a more tumour-promoting effect, although it seems likely there are also shared characteristics between these phenotypic groups.[150] Macrophages are involved in several of the hallmarks of cancer, including angiogenesis, cell invasion and migration, and suppression of antitumour immune responses.[151–153] Thus through either direct killing or inhibition of recruitment and/or modulation to promote pro-tumour responses, myeloid-derived cells are an attractive multifactorial treatment target.

The established chemotherapeutic agent trabectedin seems to have myeloid-cell-targeting properties, which were previously unidentified. The established activity of trabectedin on cancer cells is through binding to the minor groove of DNA, which disrupts the cell cycle and DNA-repair processes.[154] Trabectedin is already licensed as an orphan drug in patients with resistant soft-tissue sarcoma and ovarian cancer. For other tumour types, including in prostate,[155] breast[156] and pancreatic cancers,[157] and mesothelioma,[158] several trials investigating the effectiveness of this agent are either completed or ongoing. Data published in 2005 support a potential novel anti-inflammatory action of trabectedin on macrophages: *in vitro* studies demonstrated that trabectedin has selective cytotoxicity to human monocytes,[159] and *in vivo* data showed that the effects of trabectedin (inhibition of tumour growth and metastasis) are exclusively a result of caspase-8-induced apoptosis of monocytes and/or macrophages (including tumour-associated macrophages; TAMs) and monocyte depletion.[160] In patients with sarcoma treated with trabectedin, proof of concept was seen in the form of decreased circulating monocytes and presence of fewer TAMs in tumour-biopsy specimens.[160]

Macrophage colony-stimulating factor (CSF-1) is another emerging target involved in macrophage recruitment and survival. Extensive preclinical data on the use of CSF-1 receptor (CSF-1R) inhibitors either as a monotherapy or in combination with chemotherapy, radiation, angiogenesis inhibitors and adoptive cell transfer are currently available, thus supporting the translation of this target to clinical investigations.[161–164] Emactuzumab (also known as RG7155), a monoclonal antibody that inhibits CSF-1R, has proven clinical effectiveness and an acceptable safety profile, with data from a phase I trial demonstrating a 74% objective clinical response in patients with tenosynovial giant-cell tumours (in which CSF-1 is overexpressed).[165] This study also extended the use of emactuzumab to patients with other solid malignancies, and supported the findings that treatment reduced macrophage infiltration and promoted a more-favourable $CD8^+$:$CD4^+$ T-cell ratio within tumour tissue.[165] This proof-of-concept study[165] has led to completed[166] and ongoing[167–172] clinical trials targeting the CSF-1–CSF-1R pathway in patients with a range of solid malignancies. Interestingly, Zhu *et al.*[173] have demonstrated that in orthoptic implant models of pancreatic ductal adenocarcinoma, inhibition of CSF-1R not only enhances macrophage antigen presentation and antitumour T-cell responses but, conversely, also leads to immunosuppression by increasing expression of T-cell checkpoint molecules, such as PD-L1 and CTLA-4. Thus, by using CSF-1R blockade in conjunction with PD-L1 and CTLA-4 antagonists, these authors were able to show enhanced tumour regression.[173] This finding,[173] together with other preclinical data supporting a link between CSF-1R antagonism and T-cell function, suggests that combined innate and adaptive immunomodulatory therapies might have promising therapeutic potential.

The CD40 agonist CP-870,893 (Pfizer) is an interesting agent, which might shift macrophage activation to an antitumour phenotype, and has been tested in the phase I setting. The original aim of this monoclonal antibody therapy was to reverse the immunosuppressive actions of

TAMs by binding to and activating CD40. In the context of tumour immunology, activation of this receptor facilitates the antigen-presenting cell (APC)-mediated priming and activation of tumour-specific T cells.[174–176] In a genetically modified mouse model of pancreatic cancer, treatment with a combination of this CD40 agonist and gemcitabine resulted in tumour regression.[177] Interestingly, the mechanism of action was independent of T cells or gemcitabine: the function of TAMs was modulated, with upregulation of co-stimulatory molecules, which was consistent with a shift from a pro-tumour to an antitumour phenotype —although the tumoricidal activity was attributed predominantly to infiltrating macrophages rather than macrophages *in situ*.[177] A phase I trial of this combination therapy in treatment-naive patients with advanced-stage pancreatic ductal adenocarcinoma demonstrated an ORR of 19%.[178] Analysis of tumour biopsies detected abundant macrophages in the absence of infiltrating lymphocytes, which was in keeping with the findings of the previously reported preclinical studies, thus proving that targeting the innate immune system via immunostimulatory or immunomodulatory antibodies is an effective treatment independently of an adaptive immune response. However, without an adaptive response the development of long-term immunity and, therefore, prevention of future recurrence would be uncertain. Of note, investigators in this clinical study detected a heterogeneous response between different metastatic sites within individual patients,[178] thus reflecting the presence of tumour heterogeneity, which is well documented with use of conventional chemotherapy and targeted agents. These findings reveal that even through targeting immunity, the heterogeneity of the tumour microenvironment and stroma between a primary site and metastatic lesions will result in a variable intrapatient response.

Tasquinimod is an oral quinolone-3-carboxamide that has clear antitumour and antimetastatic properties, although an exact mechanism of action for these effects has yet to be fully elucidated; however, tasquinimod is understood to have both antiangiogenic and immunomodulatory effects.[179] One potential mechanism of action could be targeting of myeloid cells through binding to protein S100-A9, a proinflammatory protein that is known to regulate macrophage and neutrophil accumulation.[179] Thus, tasquinimod might inhibit recruitment of myeloid-derived cells to the tumour site and might also prevent binding of protein S100-A9 to two well-known proinflammatory receptors: Toll-like receptor 4 (TLR4) and MAPK/MAK/MRK overlapping kinase (also known as receptor of advanced-glycation end products). In a phase I trial investigating tasquinimod in patients with chemotherapy-naive castration-resistant prostate cancer, this agent had good clinical effectiveness in reducing the formation of new bone metastasis.[180] In the phase II setting, tasquinimod performed better than placebo, with an improved median PFS (7.6 months versus 3.3 months; $P=0.0042$) and 6-month progression-free response rate (69% versus 37%, $P<0.001$) compared with placebo alone.[181] This drug is, therefore, being tested in further clinical trials in patients with prostate cancer.[182–184]

Chemotherapy

Evidence for novel immunomodulatory effects of existing chemotherapeutic agents (such as trabectedin and tasquinimod) acting directly on innate immune cells is currently emerging. Interestingly, evidence also supports a more-complex chemotherapy-induced immune response, which is reliant on bacterial-driven inflammation. For example, germ-free or antibiotic-treated mouse models of CRC had a poor response to chemotherapy and immunotherapy (CpG-oligonucleotides) compared with mice with intact microbiota.[185] Furthermore, chemotherapy altered the composition of the microbiota in the small intestine of mice and induced translocation of bacteria to secondary lymphoid organs, where they stimulated a T-helper and T-memory immune response.[186] This response was not seen in mice under germ-free or antibiotic-treated conditions, where a reduction in T-helper response was observed and treatment resistance was demonstrated, resulting in tumour progression.[186] Thus, the chemotherapy–inflammation paradigm might be more complex than generally assumed.

Radiotherapy

As with chemotherapy, a growing body of evidence exists supporting the existence of anticancer immune response effects from treatment with radiotherapy. Radiotherapy not only directly causes the death of inflammatory cells within the stromal tissue, but also generates an acute anticancer inflammatory response. The immune system might be responsible for this 'abscopal effect' of treatment, a phenomenon whereby radiation directed at the primary tumour results in a response from distant metastases located outside the field of radiation.[187] Tumour antigenicity is promoted via recruitment of APCs to the tumour bed through 'danger signals' prompted by treatment with radiation, eventually resulting in an adaptive T-cell response.[188] Release of endogenous and exogenous TLR agonists is an example of one such danger signal. These molecules have the ability to activate dendritic cells, which can then prime a T-cell response against tumour cells.[189] This strategy has been exploited in preclinical models, through combination of a TLR agonist (CpG) with ionizing radiation, and has resulted in an enhanced tumour response in murine fibrosarcoma;[190,191] however, such a strategy remains to be evaluated in the clinical setting.

Rather than generating novel targets, a future topic of interest might be to explore further whether and how existing agents, such as chemotherapy and radiotherapy, modulate innate immunity. We might then aim to exploit these immunomodulatory effects further and in combination with, for example, checkpoint-blockade therapy.

Conclusions

Evidence increasingly suggests that inflammation and innate immunity have a vital role in tumorigenesis. Yet, as this Review has demonstrated, agents used to manipulate innate immunity in the treatment of patients with advanced-stage cancer have only shown modest results to

date, in spite of extensive and compelling experimental data from animal models. A major reason for this discrepancy might be the lack of results from clinical trials, especially in the phase II or III setting. Targeting of human cancers with these agents in phase I clinical trials might also be too late: such agents might be most effective when used either as preventative agents, perioperatively, or in patients who are in remission. The forthcoming trials investigating use of aspirin in early stage disease will be of use in answering this question.

Current approaches might also be focusing on the wrong inflammatory targets; the complexity of the inflammatory pathways means that a multitude of targets exist, and with an ever-evolving field of research, newer, more-relevant targets are likely to be identified. Importantly, targeting inflammation might only be indicated for certain tumour types, such as CRC. Thus, focusing early phase trials on certain tumour types based on the presence of inflammatory biomarkers might maximize the future potential of these agents.

Researchers and clinicians need to question why the anti-inflammatory approach has not been as successful for the treatment of advanced-stage cancer as it has been in other conditions. This lack of success could be a result of the complexity of the crosstalk and compensation that occurs within the tumour microenvironment, whereby cancerous cells are able to rapidly adapt, as discussed above in the context of crosstalk between IL-6 and EGFR signalling in ovarian cancer cells.[85]

Tumour immunotherapy is moving into an exciting era, in which targeting the adaptive immune response has proven highly effective in some patients, even when the cancer is of an advanced stage. However, this approach has not been universally effective for all tumour types and patients. The adaptive immune response is heavily dependent on innate immunity to prime T cells and to formulate a memory response. Thus, one attractive possibility might be to combine treatments that target the adaptive and innate immune pathways in the tumour microenvironment.

1. Kundu, J. K. & Surh, Y. J. Inflammation: gearing the journey to cancer. *Mutat. Res.* **659**, 15–30 (2008).
2. Mantovani, A., Allavena, P., Sica, A. & Balkwill, F. Cancer-related inflammation. *Nature* **454**, 436–444 (2008).
3. Grivennikov, S. I. & Karin, M. Inflammation and oncogenesis: a vicious connection. *Curr. Opin. Genet. Dev.* **20**, 65–71 (2010).
4. Coussens, L. M., Zitvogel, L. & Palucka, A. K. Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science* **339**, 286–291 (2013).
5. Masferrer, J. L. *et al.* Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. *Cancer Res.* **60**, 1306–1311 (2000).
6. Hwang, D., Scollard, D., Byrne, J. & Levine, E. Expression of cyclooxygenase-1 and cyclooxygenase-2 in human breast cancer. *J. Natl Cancer Inst.* **90**, 455–460 (1998).
7. Gupta, S., Srivastava, M., Ahmad, N., Bostwick, D. G. & Mukhtar, H. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. *Prostate* **42**, 73–78 (2000).
8. Tucker, O. N. *et al.* Cyclooxygenase-2 expression is up-regulated in human pancreatic cancer. *Cancer Res.* **59**, 987–990 (1999).
9. Buckman, S. Y. *et al.* COX-2 expression is induced by UVB exposure in human skin: implications for the development of skin cancer. *Carcinogenesis* **19**, 723–729 (1998).
10. Wolff, H. *et al.* Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res.* **58**, 4997–5001 (1998).
11. Mohammed, S. I. *et al.* Expression of cyclooxygenase-2 (COX-2) in human invasive transitional cell carcinoma (TCC) of the urinary bladder. *Cancer Res.* **59**, 5647–5650 (1999).
12. Chan, G. *et al.* Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. *Cancer Res.* **59**, 991–994 (1999).
13. Waddell, W. R. & Loughry, R. W. Sulindac for polyposis of the colon. *J. Surg. Oncol.* **24**, 83–87 (1983).
14. Algra, A. M. & Rothwell, P. M. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. *Lancet Oncol.* **13**, 518–527 (2012).
15. Rothwell, P. M. *et al.* Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. *Lancet* **379**, 1602–1612 (2012)
16. Rothwell, P. M. *et al.* Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet* **379**, 1591–1601 (2012).
17. Cuzick, J. *et al.* Estimates of benefits and harms of prophylactic use of aspirin in the general population. *Ann. Oncol.* **26**, 47–57 (2015).
18. Gay, L. J. & Felding-Habermann, B. Contribution of platelets to tumour metastasis. *Nat. Rev. Cancer* **11**, 123–134 (2011).
19. Eberhart, C. E. *et al.* Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* **107**, 1183–1188 (1994).
20. Gierach, G. L. *et al.* Nonsteroidal anti-inflammatory drugs and breast cancer risk in the National Institutes of Health–AARP Diet and Health Study. *Breast Cancer Res.* **10**, R38 (2008).
21. Salinas, C. A. *et al.* Use of aspirin and other nonsteroidal antiinflammatory medications in relation to prostate cancer risk. *Am. J. Epidemiol.* **172**, 578–590 (2010).
22. Liao, X. *et al.* Aspirin use, tumor *PIK3CA* mutation, and colorectal-cancer survival. *N. Engl. J. Med.* **367**, 1596–1606 (2012).
23. Fraser, D. M., Sullivan, F. M., Thompson, A. M. & McCowan, C. Aspirin use and survival after the diagnosis of breast cancer: a population-based cohort study. *Br. J. Cancer* **111**, 623–627 (2014).
24. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT00003835?term=NCT00003835&rank=1 (2013).
25. Ng, K. *et al.* Aspirin and COX-2 inhibitor use in patients with stage III colon cancer. *J. Natl Cancer Inst.* **107**, 345 (2015).
26. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01150045 (2013).
27. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT00565708?term=NCT00565708&rank=1 (2015).
28. The Add Aspirin trial. *addaspirintrial.org* [online], http://www.addaspirintrial.org/ (2015).
29. Dulak, J. & Jozkowicz, A. Anti-angiogenic and anti inflammatory effects of statins: relevance to anti-cancer therapy. *Curr. Cancer Drug Targets* **5**, 579–594 (2005).
30. Boudreau, D. M., Yu, O. & Johnson, J. Statin use and cancer risk: a comprehensive review. *Expert Opin. Drug Saf.* **9**, 603–621 (2010).
31. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02161822?term=NCT02161822&rank=1 (2014).
32. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01821404?term=NCT01821404&rank=1 (2015).
33. Argiles, J. M., Busquets, S., Toledo, M. & Lopez-Soriano, F. J. The role of cytokines in cancer cachexia. *Curr. Opin. Support. Palliat. Care* **3**, 263–268 (2009).
34. Trajkovic-Vidakovic, M., de Graeff, A., Voest, E. E. & Teunissen, S. C. Symptoms tell it all: a systematic review of the value of symptom assessment to predict survival in advanced cancer patients. *Crit. Rev. Oncol. Hematol.* **84**, 130–148 (2012).
35. Leggas, M. *et al.* Intensive anti-inflammatory therapy with dexamethasone in patients with non-small cell lung cancer: effect on chemotherapy toxicity and efficacy. *Cancer Chemother. Pharmacol.* **63**, 731–743 (2009).
36. Hanahan, D. & Weinberg, R. A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
37. Germano, G., Allavena, P. & Mantovani, A. Cytokines as a key component of cancer-related inflammation. *Cytokine* **43**, 374–379, (2008).
38. Hanahan, D. & Coussens, L. M. Accessories to the crime: functions of cells recruited to the tumor microenvironment. *Cancer Cell* **21**, 309–322 (2012).
39. Argiles, J. M., Moore-Carrasco, R., Fuster, G., Busquets, S. & Lopez-Soriano, F. J. Cancer cachexia: the molecular mechanisms. *Int. J. Biochem. Cell Biol.* **35**, 405–409 (2003).
40. Szlosarek, P., Charles, K. A. & Balkwill, F. R. Tumour necrosis factor-alpha as a tumour promoter. *Eur. J. Cancer* **42**, 745–750 (2006).

41. Feldmann, M. & Maini, R. N. Anti-TNF therapy, from rationale to standard of care: what lessons has it taught us? *J. Immunol.* **185**, 791–794 (2010).
42. Sandborn, W. J. & Hanauer, S. B. Antitumor necrosis factor therapy for inflammatory bowel disease: a review of agents, pharmacology, clinical results, and safety. *Inflamm. Bowel Dis.* **5**, 119–133 (1999).
43. Knight, D. M. *et al.* Construction and initial characterization of a mouse–human chimeric anti-TNF antibody. *Mol. Immunol.* **30**, 1443–1453 (1993).
44. Mohler, K. M. *et al.* Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J. Immunol.* **151**, 1548–1561 (1993).
45. Wiens, A., Correr, C. J., Venson, R., Otuki, M. F. & Pontarolo, R. A systematic review and meta-analysis of the efficacy and safety of adalimumab for treating rheumatoid arthritis. *Rheumatol. Int.* **30**, 1063–1070 (2010).
46. Carswell, E. A. *et al.* An endotoxin-induced serum factor that causes necrosis of tumors. *Proc. Natl Acad. Sci. USA* **72**, 3666–3670 (1975).
47. Balkwill, F. Tumour necrosis factor and cancer. *Nat. Rev. Cancer* **9**, 361–371 (2009).
48. Szlosarek, P. W. *et al.* Expression and regulation of tumor necrosis factor alpha in normal and malignant ovarian epithelium. *Mol. Cancer Ther.* **5**, 382–390 (2006).
49. Miles, D. W. *et al.* Expression of tumour necrosis factor (TNF alpha) and its receptors in benign and malignant breast tissue. *Int. J. Cancer* **56**, 777–782 (1994).
50. Balkwill, F. TNF-alpha in promotion and progression of cancer. *Cancer Metastasis Rev.* **25**, 409–416 (2006).
51. Harrison, M. L. *et al.* Tumor necrosis factor alpha as a new target for renal cell carcinoma: two sequential phase II trials of infliximab at standard and high dose. *J. Clin. Oncol.* **25**, 4542–4549 (2007).
52. Larkin, J. M. *et al.* A phase I/II trial of sorafenib and infliximab in advanced renal cell carcinoma. *Br. J. Cancer* **103**, 1149–1153 (2010).
53. Wu, C. *et al.* Disrupting cytokine signaling in pancreatic cancer: a phase I/II study of etanercept in combination with gemcitabine in patients with advanced disease. *Pancreas* **42**, 813–818 (2013).
54. Sheen-Chen, S. M., Chen, W. J., Eng, H. L. & Chou, F. F. Serum concentration of tumor necrosis factor in patients with breast cancer. *Breast Cancer Res. Treat.* **43**, 211–215 (1997).
55. Madhusudan, S. *et al.* A phase II study of etanercept (Enbrel), a tumor necrosis factor alpha inhibitor in patients with metastatic breast cancer. *Clin. Cancer Res.* **10**, 6528–6534 (2004).
56. Naylor, M. S., Stamp, G. W., Foulkes, W. D., Eccles, D. & Balkwill, F. R. Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. *J. Clin. Invest.* **91**, 2194–2206 (1993).
57. Hassan, M. I., Kassim, S. K., Saeda, L., Laban, M. & Khalifa, A. Ovarian cancer-induced immunosuppression: relationship to tumor necrosis factor-alpha (TNF-alpha) release from ovarian tissue. *Anticancer Res.* **19**, 5657–5662 (1999).
58. Madhusudan, S. *et al.* Study of etanercept, a tumor necrosis factor-alpha inhibitor, in recurrent ovarian cancer. *J. Clin. Oncol.* **23**, 5950–5959 (2005).
59. Wolfe, F. & Michaud, K. Biologic treatment of rheumatoid arthritis and the risk of malignancy: analyses from a large US observational study. *Arthritis Rheum.* **56**, 2886–2895 (2007).
60. Wolfe, F. & Michaud, K. The effect of methotrexate and anti-tumor necrosis factor therapy on the risk of lymphoma in rheumatoid arthritis in 19,562 patients during 89,710 person-years of observation. *Arthritis Rheum.* **56**, 1433–1439 (2007).
61. Askling, J. *et al.* Haematopoietic malignancies in rheumatoid arthritis: lymphoma risk and characteristics after exposure to tumour necrosis factor antagonists. *Ann. Rheum. Dis.* **64**, 1414–1420, (2005).
62. Askling, J. *et al.* Risks of solid cancers in patients with rheumatoid arthritis and after treatment with tumour necrosis factor antagonists. *Ann. Rheum. Dis.* **64**, 1421–1426 (2005).
63. Mariette, X. *et al.* Malignancies associated with tumour necrosis factor inhibitors in registries and prospective observational studies: a systematic review and meta-analysis. *Ann. Rheum. Dis.* **70**, 1895–1904 (2011).
64. Leombruno, J. P., Einarson, T. R. & Keystone, E. C. The safety of anti-tumour necrosis factor treatments in rheumatoid arthritis: meta and exposure-adjusted pooled analyses of serious adverse events. *Ann. Rheum. Dis.* **68**, 1136–1145 (2009).
65. Askling, J. *et al.* Cancer risk with tumor necrosis factor alpha (TNF) inhibitors: meta-analysis of randomized controlled trials of adalimumab, etanercept, and infliximab using patient level data. *Pharmacoepidemiol. Drug Saf.* **20**, 119–130 (2011).
66. Moulis, G. *et al.* Cancer risk of anti-TNF-alpha at recommended doses in adult rheumatoid arthritis: a meta-analysis with intention to treat and per protocol analyses. *PLoS ONE* **7**, e48991 (2012).
67. Taniguchi, K. & Karin, M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin. Immunol.* **26**, 54–74 (2014).
68. Ancrile, B., Lim, K. H. & Counter, C. M. Oncogenic RAS-induced secretion of IL6 is required for tumorigenesis. *Genes Dev.* **21**, 1714–1719 (2007).
69. Yonish-Rouach, E. *et al.* Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* **352**, 345–347 (1991).
70. Stone, R. L. *et al.* Paraneoplastic thrombocytosis in ovarian cancer. *N. Engl. J. Med.* **366**, 610–618 (2012).
71. Kulbe, H. *et al.* The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells. *Cancer Res.* **67**, 585–592 (2007).
72. Anglesio, M. S. *et al.* IL6–STAT3–HIF signaling and therapeutic response to the angiogenesis inhibitor sunitinib in ovarian clear cell cancer. *Clin. Cancer Res.* **17**, 2538–2548 (2011).
73. Shain, K. H. *et al.* β1 integrin adhesion enhances IL-6-mediated STAT3 signaling in myeloma cells: implications for microenvironment influence on tumor survival and proliferation. *Cancer Res.* **69**, 1009–1015 (2009).
74. Naugler, W. E. *et al.* Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. *Science* **317**, 121–124 (2007).
75. van Zaanen, H. C. *et al.* Chimaeric anti-interleukin 6 monoclonal antibodies in the treatment of advanced multiple myeloma: a phase I dose-escalating study. *Br. J. Haematol.* **102**, 783–790 (1998).
76. Kurzrock, R. *et al.* A phase I, open-label study of siltuximab, an anti-IL-6 monoclonal antibody, in patients with B-cell non-Hodgkin lymphoma, multiple myeloma, or Castleman disease. *Clin. Cancer Res.* **19**, 3659–3670 (2013).
77. Orlowski, R. Z. *et al.* A phase 2, randomized, double-blind, placebo-controlled study of siltuximab (anti-IL-6 mAb) and bortezomib versus bortezomib alone in patients with relapsed or refractory multiple myeloma. *Am. J. Hematol.* **90**, 42–49 (2015
78. Coward, J. *et al.* Interleukin-6 as a therapeutic target in human ovarian cancer. *Clin. Cancer Res.* **17**, 6083–6096 (2011).
79. Dorff, T. B. *et al.* Clinical and correlative results of SWOG S0354: a phase II trial of CNTO328 (siltuximab), a monoclonal antibody against interleukin-6, in chemotherapy-pretreated patients with castration-resistant prostate cancer. *Clin. Cancer Res.* **16**, 3028–3034 (2010).
80. Borsellino, N., Belldegrun, A. & Bonavida, B. Endogenous interleukin 6 is a resistance factor for cis-diamminedichloroplatinum and etoposide-mediated cytotoxicity of human prostate carcinoma cell lines. *Cancer Res.* **55**, 4633–4639 (1995).
81. Hudes, G. *et al.* A phase 1 study of a chimeric monoclonal antibody against interleukin-6, siltuximab, combined with docetaxel in patients with metastatic castration-resistant prostate cancer. *Invest. New Drugs* **31**, 669–676 (2013).
82. Fizazi, K. *et al.* Randomised phase II study of siltuximab (CNTO 328), an anti-IL-6 monoclonal antibody, in combination with mitoxantrone/prednisone versus mitoxantrone/prednisone alone in metastatic castration-resistant prostate cancer. *Eur. J. Cancer* **48**, 85–93 (2012).
83. Rossi, J. F. *et al.* A phase I/II study of siltuximab (CNTO 328), an anti-interleukin-6 monoclonal antibody, in metastatic renal cell cancer. *Br. J. Cancer* **103**, 1154–1162 (2010).
84. Ando, K. *et al.* Possible role for tocilizumab, an anti-interleukin-6 receptor antibody, in treating cancer cachexia. *J. Clin. Oncol.* **31**, e69–e72 (2013).
85. Milagre, C. S. *et al.* Adaptive upregulation of EGFR limits attenuation of tumor growth by neutralizing IL-6 antibodies with implications for combined therapy in ovarian cancer. *Cancer Res.* **75**, 1255–1264 (2015).
86. Zhang, J., Patel, L. & Pienta, K. J. Targeting chemokine (C-C motif) ligand 2 (CCL2) as an example of translation of cancer molecular biology to the clinic. *Prog. Mol. Biol. Transl. Sci.* **95**, 31–53 (2010).
87. Sandhu, S. K. *et al.* A first-in-human, first-in-class, phase I study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 in patients with solid tumors. *Cancer Chemother. Pharmacol.* **71**, 1041–1050 (2013).
88. Pienta, K. J. *et al.* Phase 2 study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 (CCL2), in metastatic castration-resistant prostate cancer. *Invest. New Drugs* **31**, 760–768 (2013).
89. Bonapace, L. *et al.* Cessation of CCL2 inhibition accelerates breast cancer metastasis by promoting angiogenesis. *Nature* **515**, 130–133 (2014).
90. Tomimatsu, S., Ichikura, T. & Mochizuki, H. Significant correlation between expression of interleukin-1α and liver metastasis in gastric carcinoma. *Cancer* **91**, 1272–1276 (2001).
91. Singer, C. F. *et al.* Interleukin 1 system and sex steroid receptor expression in human breast cancer: interleukin 1α protein secretion is correlated with malignant phenotype. *Clin. Cancer Res.* **9**, 4877–4883 (2003).

92. Ling, J. *et al.* KrasG12D-induced IKK2/β/NF-κB activation by IL-1α and p62 feedforward loops is required for development of pancreatic ductal adenocarcinoma. *Cancer Cell* **21**, 105–120 (2012).
93. Hong, D. S. *et al.* MABp1, a first-in-class true human antibody targeting interleukin-1α in refractory cancers: an open-label, phase 1 dose-escalation and expansion study. *Lancet Oncol.* **15**, 656–666 (2014).
94. Oppenheim, J. J., Zachariae, C. O., Mukaida, N. & Matsushima, K. Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu. Rev. Immunol.* **9**, 617–648 (1991).
95. Larsen, C. G., Anderson, A. O., Appella, E., Oppenheim, J. J. & Matsushima, K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science* **243**, 1464–1466 (1989).
96. Schroder, J. M., Sticherling, M., Henneicke, H. H., Preissner, W. C. & Christophers, E. IL-1 alpha or tumor necrosis factor-alpha stimulate release of three NAP-1/IL-8-related neutrophil chemotactic proteins in human dermal fibroblasts. *J. Immunol.* **144**, 2223–2232 (1990).
97. Krieger, M. *et al.* Activation of human basophils through the IL-8 receptor. *J. Immunol.* **149**, 2662–2667 (1992).
98. Wang, J. M., Taraboletti, G., Matsushima, K., Van Damme, J. & Mantovani, A. Induction of haptotactic migration of melanoma cells by neutrophil activating protein/interleukin-8. *Biochem. Biophys. Res. Commun.* **169**, 165–170 (1990).
99. Strieter, R. M. *et al.* Interleukin-8. A corneal factor that induces neovascularization. *Am. J. Pathol.* **141**, 1279–1284 (1992).
100. Koch, A. E. *et al.* Interleukin-8 as a macrophage-derived mediator of angiogenesis. *Science* **258**, 1798–1801 (1992).
101. Schadendorf, D. *et al.* IL-8 produced by human malignant melanoma cells *in vitro* is an essential autocrine growth factor. *J. Immunol.* **151**, 2667–2675 (1993).
102. Sanmamed, M. F. *et al.* Serum interleukin-8 reflects tumor burden and treatment response across malignancies of multiple tissue origins. *Clin. Cancer Res.* **20**, 5697–5707 (2014).
103. Lee, L. F. *et al.* IL-8 reduced tumorigenicity of human ovarian cancer *in vivo* due to neutrophil infiltration. *J. Immunol.* **164**, 2769–2775 (2000).
104. Lee, L. F. *et al.* Taxol-dependent transcriptional activation of IL-8 expression in a subset of human ovarian cancer. *Cancer Res.* **56**, 1303–1308 (1996).
105. Bekes, E. M. *et al.* Tumor-recruited neutrophils and neutrophil TIMP-free MMP-9 regulate coordinately the levels of tumor angiogenesis and efficiency of malignant cell intravasation. *Am. J. Pathol.* **179**, 1455–1470 (2011).
106. Huang, S. *et al.* Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. *Am. J. Pathol.* **161**, 125–134 (2002).
107. Zhu, Y. M., Webster, S. J., Flower, D. & Woll, P. J. Interleukin-8/CXCL8 is a growth factor for human lung cancer cells. *Br. J. Cancer* **91**, 1970–1976 (2004).
108. Matsuo, Y. *et al.* CXC-chemokine/CXCR2 biological axis promotes angiogenesis *in vitro* and *in vivo* in pancreatic cancer. *Int. J. Cancer* **125**, 1027–1037 (2009).
109. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT00003835?term=NCT00003835&rank=1 (2013).
110. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02001974 (2015).
111. Ginestier, C. *et al.* CXCR1 blockade selectively targets human breast cancer stem cells *in vitro* and in xenografts. *J. Clin. Invest.* **120**, 485–497 (2010).
112. Müller, A. *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**, 50–56 (2001).
113. Hall, J. M. & Korach, K. S. Stromal cell-derived factor 1, a novel target of estrogen receptor action, mediates the mitogenic effects of estradiol in ovarian and breast cancer cells. *Mol. Endocrinol.* **17**, 792–803 (2003).
114. Taichman, R. S. *et al.* Use of the stromal cell-derived factor-1/CXCR4 pathway in prostate cancer metastasis to bone. *Cancer Res.* **62**, 1832–1837 (2002).
115. Zagzag, D. *et al.* Stromal cell-derived factor-1alpha and CXCR4 expression in hemangioblastoma and clear cell-renal cell carcinoma: von Hippel-Lindau loss-of-function induces expression of a ligand and its receptor. *Cancer Res.* **65**, 6178–6188 (2005).
116. Kim, S. Y. *et al.* Inhibition of the CXCR4/CXCL12 chemokine pathway reduces the development of murine pulmonary metastases. *Clin. Exp. Metastases* **25**, 201–211 (2008).
117. Geminder, H. *et al.* A possible role for CXCR4 and its ligand, the CXC chemokine stromal cell-derived factor-1, in the development of bone marrow metastases in neuroblastoma. *J. Immunol.* **167**, 4747–4757 (2001).
118. Orimo, A. *et al.* Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**, 335–348 (2005).
119. Stuelten, C. H. *et al.* Breast cancer cells induce stromal fibroblasts to express MMP-9 via secretion of TNF-α and TGF-β. *J. Cell Sci.* **118**, 2143–2153 (2005).
120. Jung, Y. *et al.* Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. *Nat. Commun.* **4**, 1795 (2013).
121. Smith, M. C. *et al.* CXCR4 regulates growth of both primary and metastatic breast cancer. *Cancer Res.* **64**, 8604–8612 (2004).
122. Kim, M. *et al.* CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. *Cancer Res.* **70**, 10411–10421 (2010).
123. D'Alterio, C. *et al.* Inhibition of stromal CXCR4 impairs development of lung metastases. *Cancer Immunol. Immunother.* **61**, 1713–1720 (2012).
124. Uchida, D. *et al.* Blockade of CXCR4 in oral squamous cell carcinoma inhibits lymph node metastases. *Eur. J. Cancer* **47**, 452–459 (2011).
125. Kajiyama, H. *et al.* Involvement of SDF-1α/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. *Int. J. Cancer* **122**, 91–99 (2008).
126. Feig, C. *et al.* Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc. Natl Acad. Sci. USA* **110**, 20212–20217 (2013).
127. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02179970 (2015).
128. Bromberg, J. STAT proteins and oncogenesis. *J. Clin. Invest.* **109**, 1139–1142 (2002).
129. Verstovsek, S. *et al.* A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N. Engl. J. Med.* **366**, 799–807 (2012).
130. Harrison, C. *et al.* JAK inhibition with ruxolitinib versus best available therapy for myelofibrosis. *N. Engl. J. Med.* **366**, 787–798 (2012).
131. Younes, A. *et al.* Phase I study of a novel oral Janus kinase 2 inhibitor, SB1518, in patients with relapsed lymphoma: evidence of clinical and biologic activity in multiple lymphoma subtypes. *J. Clin. Oncol.* **30**, 4161–4167 (2012).
132. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02055781?term=pacritinib&rank=3 (2015).
133. Gao, S. P. *et al.* Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. *J. Clin. Invest.* **117**, 3846–3856 (2007).
134. Song, L., Rawal, B., Nemeth, J. A. & Haura, E. B. JAK1 activates STAT3 activity in non-small-cell lung cancer cells and IL-6 neutralizing antibodies can suppress JAK1-STAT3 signaling. *Mol. Cancer Ther.* **10**, 481–494 (2011).
135. Karin, M. Nuclear factor-kappaB in cancer development and progression. *Nature* **441**, 431–436 (2006).
136. DiDonato, J. A., Mercurio, F. & Karin, M. NF-kappaB and the link between inflammation and cancer. *Immunol. Rev.* **246**, 379–400 (2012).
137. San Miguel, J. F. *et al.* Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. *N. Engl. J. Med.* **359**, 906–917 (2008).
138. San Miguel, J. F. *et al.* Persistent overall survival benefit and no increased risk of second malignancies with bortezomib–melphalan–prednisone versus melphalan–prednisone in patients with previously untreated multiple myeloma. *J. Clin. Oncol.* **31**, 448–455 (2013).
139. Goy, A. *et al.* Bortezomib in patients with relapsed or refractory mantle cell lymphoma: updated time-to-event analyses of the multicenter phase 2 PINNACLE study. *Ann. Oncol.* **20**, 520–525 (2009).
140. Hata, H. *et al.* Interleukin-6 gene expression in multiple myeloma: a characteristic of immature tumor cells. *Blood* **81**, 3357–3364 (1993).
141. Orlowski, R. Z. *et al.* Phase II, randomized, double blind, placebo-controlled study comparing siltuximab plus bortezomib versus bortezomib alone in pts with relapsed/refractory multiple myeloma [abstract]. *J. Clin. Oncol.* **30** (Suppl.), a8018 (2012).
142. San-Miguel, J. *et al.* Phase 2 randomized study of bortezomib-melphalan-prednisone with or without siltuximab (anti-IL-6) in multiple myeloma. *Blood* **123**, 4136–4142 (2014).
143. Bindea, G. *et al.* Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. *Immunity* **39**, 782–795 (2013).
144. Fridman, W. H., Pages, F., Sautes-Fridman, C. & Galon, J. The immune contexture in human tumours: impact on clinical outcome. *Nat. Rev. Cancer* **12**, 298–306 (2012).
145. Brahmer, J. R. *et al.* Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J. Clin. Oncol.* **28**, 3167–3175 (2010).
146. Topalian, S. L. *et al.* Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* **366**, 2443–2454 (2012).
147. Wolchok, J. D. *et al.* Nivolumab plus ipilimumab in advanced melanoma. *N. Engl. J. Med.* **369**, 122–133 (2013).
148. Powles, T. *et al.* MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* **515**, 558–562 (2014).

149. Bingle, L., Brown, N. J. & Lewis, C. E. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J. Pathol.* **196**, 254–265 (2002).
150. Murray, P. J. *et al.* Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* **41**, 14–20 (2014).
151. Condeelis, J. & Pollard, J. W. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* **124**, 263–266 (2006).
152. Pollard, J. W. Tumour-educated macrophages promote tumour progression and metastasis. *Nat. Rev Cancer* **4**, 71–78 (2004).
153. Gabrilovich, D. I., Ostrand-Rosenberg, S. & Bronte, V. Coordinated regulation of myeloid cells by tumours. *Nat. Rev. Immunol.* **12**, 253–268 (2012).
154. D'Incalci, M. *et al.* Unique features of the mode of action of ET-743. *Oncologist* **7**, 210–216 (2002).
155. Michaelson, M. D. *et al.* Multicenter phase II study of trabectedin in patients with metastatic castration-resistant prostate cancer. *Ann. Oncol.* **23**, 1234–1240 (2012).
156. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT00050427?term=NCT00050427&rank=1 (2011).
157. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01339754?term=NCT01339754&rank=1 (2014).
158. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02194231?term=NCT02194231&rank=1 (2015).
159. Allavena, P. *et al.* Anti-inflammatory properties of the novel antitumor agent yondelis (trabectedin): inhibition of macrophage differentiation and cytokine production. *Cancer Res.* **65**, 2964–2971 (2005).
160. Germano, G. *et al.* Role of macrophage targeting in the antitumor activity of trabectedin. *Cancer Cell* **23**, 249–262 (2013).
161. Mitchem, J. B. *et al.* Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. *Cancer Res.* **73**, 1128–1141 (2013).
162. Xu, J. *et al.* CSF1R signaling blockade stanches tumor-infiltrating myeloid cells and improves the efficacy of radiotherapy in prostate cancer. *Cancer Res.* **73**, 2782–2794 (2013).
163. Priceman, S. J. *et al.* Targeting distinct tumor-infiltrating myeloid cells by inhibiting CSF-1-receptor: combating tumor evasion of antiangiogenic therapy. *Blood* **115**, 1461–1471 (2010).
164. Mok, S. *et al.* Inhibition of CSF-1 receptor improves the antitumor efficacy of adoptive cell transfer immunotherapy. *Cancer Res.* **74**, 153–161 (2014).
165. Ries, C. H. *et al.* Targeting tumor-associated macrophages with anti-CSF-1R antibody reveals a strategy for cancer therapy. *Cancer Cell* **25**, 846–859 (2014).
166. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01444404?term=NCT01444404&rank=1 (2014).
167. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01346358?term=NCT01346358&rank=1 (2014).
168. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01596751?term=NCT01596751&rank=1 (2015).
169. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02265536?term=NCT02265536&rank=1 (2015).
170. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01349036?term=NCT01349036&rank=1 (2015).
171. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01004861?term=NCT01004861&rank=1 (2015).
172. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01346358?term=NCT01346358&rank=1 (2014).
173. Zhu, Y. *et al.* CSF1/CSF1R blockade reprograms tumor-infiltrating macrophages and improves response to T-cell checkpoint immunotherapy in pancreatic cancer models. *Cancer Res.* **74**, 5057–5069 (2014).
174. Bennett, S. R. *et al.* Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* **393**, 478–480 (1998).
175. Sotomayor, E. M. *et al.* Conversion of tumor-specific $CD4^+$ T-cell tolerance to T-cell priming through *in vivo* ligation of CD40. *Nat. Med.* **5**, 780–787 (1999).
176. Diehl, L. *et al.* CD40 activation *in vivo* overcomes peptide-induced peripheral cytotoxic T-lymphocyte tolerance and augments anti-tumor vaccine efficacy. *Nat. Med.* **5**, 774–779 (1999).
177. Beatty, G. L. *et al.* CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* **331**, 1612–1616 (2011).
178. Beatty, G. L. *et al.* A phase I study of an agonist CD40 monoclonal antibody (CP-870,893) in combination with gemcitabine in patients with advanced pancreatic ductal adenocarcinoma. *Clin. Cancer Res.* **19**, 6286–6295 (2013).
179. Bjork, P. *et al.* Identification of human S100A9 as a novel target for treatment of autoimmune disease via binding to quinoline-3-carboxamides. *PLoS Biol.* **7**, e97 (2009).
180. Bratt, O. *et al.* Open-label, clinical phase I studies of tasquinimod in patients with castration-resistant prostate cancer. *Br. J. Cancer* **101**, 1233–1240 (2009).
181. Pili, R. *et al.* Phase II randomized, double-blind, placebo-controlled study of tasquinimod in men with minimally symptomatic metastatic castrate-resistant prostate cancer. *J. Clin. Oncol.* **29**, 4022–4028 (2011).
182. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01234311?term=NCT+01234311&rank=1 (2014).
183. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01732549?term=NCT+01732549&rank=1 (2014).
184. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01513733?term=NCT+01513733&rank=1 (2014).
185. Iida, N. *et al.* Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. *Science* **342**, 967–970 (2013).
186. Viaud, S. *et al.* The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* **342**, 971–976 (2013).
187. Demaria, S. *et al.* Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int. J. Radiat. Oncol. Biol. Phys.* **58**, 862–870 (2004).
188. Burnette, B. & Weichselbaum, R. R. Radiation as an immune modulator. *Semin. Radiat. Oncol.* **23**, 273–280 (2013).
189. Roses, R. E., Datta, J. & Czerniecki, B. J. Radiation as immunomodulator: implications for dendritic cell-based immunotherapy. *Radiat. Res.* **182**, 211–218, (2014).
190. Milas, L. *et al.* CpG oligodeoxynucleotide enhances tumor response to radiation. *Cancer Res.* **64**, 5074–5077 (2004).
191. Mason, K. A. *et al.* Targeting toll-like receptor 9 with CpG oligodeoxynucleotides enhances tumor response to fractionated radiotherapy. *Clin. Cancer Res.* **11**, 361–369 (2005).
192. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01637532?term=NCT01637532&rank=1 (2014).
193. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01767857?term=NCT01767857&rank=1 (2015).
194. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02001974?term=NCT02001974&rank=1 (2015).
195. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02179970?term=NCT02179970&rank=1 (2015).
196. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02055781?term=NCT02055781&rank=1 (2015).
197. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02277093?term=NCT02277093&rank=1 (2015).
198. Demetri, G. D. *et al.* Efficacy and safety of trabectedin in patients with advanced or metastatic liposarcoma or leiomyosarcoma after failure of prior anthracyclines and ifosfamide: results of a randomized phase II study of two different schedules. *J. Clin. Oncol.* **27**, 4188–4196 (2009).
199. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT00002904?term=NCT00002904&rank=1 (2012).
200. US National Library Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01346358?term=NCT01346358&rank=1 (2014).
201. US National Library Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01444404?term=NCT01444404&rank=1 (2015).
202. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01004861?term=NCT01004861&rank=1 (2015).
203. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01234311?term=NCT01234311&rank=1 (2014).

Acknowledgements

S.M.C. gratefully acknowledges research funding from Cancer Research UK. F.R.B. gratefully acknowledges research funding from Cancer Research UK (grant C587/A16354) and the European Research Council (grant GA 322,566).

Author contributions

S.M.C. and F.R.B. contributed equally to researching data for the article, discussions of article content, writing the article, and reviewing and/or editing of the manuscript before submission.

Exhibit 67

Published in final edited form as:
Free Radic Biol Med. 2010 December 1; 49(11): 1603–1616. doi:10.1016/j.freeradbiomed.2010.09.006.

Oxidative stress, inflammation, and cancer: How are they linked?

Simone Reuter, **Subash C. Gupta**, **Madan M. Chaturvedi**, and **Bharat B. Aggarwal**
Cytokine Research Laboratory, Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

Abstract

Extensive research during last two decades has revealed the mechanism by which continued oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases including cancer, diabetes, cardiovascular, neurological and pulmonary diseases. Oxidative stress can activate a variety of transcription factors including NF-κB, AP-1, p53, HIF-1α, PPAR-γ, β-catenin/Wnt, and Nrf2. Activation of these transcription factors can lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules, and anti-inflammatory molecules. How oxidative stress activates inflammatory pathways leading to transformation of a normal cell to tumor cell, tumor cell survival, proliferation, chemoresistance, radioresistance, invasion, angiogenesis and stem cell survival is the focus of this review. Overall, observations to date suggest that oxidative stress, chronic inflammation, and cancer are closely linked.

Keywords

Oxidative stress; Inflammation; Cancer; Pro-oxidants; Anti-oxidants; NF-κB

1. Introduction

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism [1]. ROS are products of a normal cellular metabolism and play vital roles in stimulation of signaling pathways in plant and animal cells in response to changes of intra- and extracellular environmental conditions [2]. Most ROS are generated in cells by the mitochondrial respiratory chain [3]. During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH•), and organic peroxides as normal products of the biological reduction of molecular oxygen [4]. The electron transfer to molecular oxygen occurs at the level of the respiratory chain, and

Address correspondence to: Bharat B. Aggarwal, Ph.D., Cytokine Research Laboratory, Department of Experimental Therapeutics (Unit 0143), The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA, aggarwal@mdanderson.org; Tel: +1 713-794-1817; Fax: +1-713-745-6339.

Conflict of interest: The authors declare that they have no conflict of interest.

the electron transport chains are located in membranes of the mitochondria [5,6]. Under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO), which can generate other reactive nitrogen species (RNS) [3]. RNS can further generate other reactive species, e.g., reactive aldehydes-malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), by inducing excessive lipid peroxidation [7]. Proteins and lipids are also significant targets for oxidative attack, and modification of these molecules can increase the risk of mutagenesis [8].

Under a sustained environmental stress, ROS are produced over a long time, and thus significant damage may occur to cell structure and functions and may induce somatic mutations and neoplastic transformation [9,10]. Indeed, cancer initiation and progression has been linked to oxidative stress by increasing DNA mutations or inducing DNA damage, genome instability, and cell proliferation [11].

The skin, for example, is chronically exposed to both endogenous and environmental pro-oxidants due to its interface function between the body and the environment, and to protect the skin against this overload of oxidant species, it needs a well-organized system of both chemical and enzymatic antioxidants [12]. The lungs, which are directly exposed to oxygen concentrations higher than in most other tissues, are protected against these oxidants by a variety of antioxidant mechanisms [13]. Furthermore, aging, which is considered as an impairment of body functions over time, caused by the accumulation of molecular damage in DNA, proteins and lipids, is also characterized by an increase in intracellular oxidative stress due to the progressive decrease of the intracellular ROS scavenging [14]. Acting to protect the organism against these harmful pro-oxidants is a complex system of enzymatic antioxidants [e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, catalase] and nonenzymatic antioxidants [e.g., glutathione (GSH), vitamins C and D] [15] (Figure 1).

ROS are involved in a wide spectrum of diseases, including chronic inflammation (Table 1), and in a wide variety of different cancers (Table 2).

Chronic inflammation is induced by biological, chemical, and physical factors and is in turn associated with an increased risk of several human cancers [54]. The link between inflammation and cancer has been suggested by epidemiological and experimental data [55,56] and confirmed by anti-inflammatory therapies that show efficacy in cancer prevention and treatment [57]. The fact that continuous irritation over long periods of time can lead to cancer had already been described in the traditional Ayurvedic (meaning, the science of long life) medical system, written as far back as 5000 years ago [58]. Whether this irritation is the same as what Rudolf Virchow referred to as inflammation in the nineteenth century is uncertain [59]. Virchow first noted that inflammatory cells are present within tumors and that tumors arise at sites of chronic inflammation [60]. This inflammation is now regarded as a “secret killer” for diseases such as cancer. For example, inflammatory bowel diseases such as Crohn's disease and ulcerative colitis are associated with increased risk of colon adenocarcinoma [61-63], and chronic pancreatitis is related to an increased rate of pancreatic cancer [64].

The exact mechanisms by which a wound-healing process turns into cancer are topics of intense research [57,65], and possible mechanisms include induction of genomic instability, alterations in epigenetic events and subsequent inappropriate gene expression, enhanced proliferation of initiated cells, resistance to apoptosis, aggressive tumor neo-vascularization, invasion through tumor-associated basement membrane, and metastasis [66]. How oxidative stress modulates these different stages of inflammation-induced carcinogenesis is the focus of this review.

2. Inflammatory network

The sources of inflammation are widespread and include microbial and viral infections, exposure to allergens, radiation and toxic chemicals, autoimmune and chronic diseases, obesity, consumption of alcohol, tobacco use, and a high-calorie diet [60,67]. In general, the longer the inflammation persists, the higher the risk of cancer. Two stages of inflammation exist, acute and chronic inflammation. Acute inflammation is an initial stage of inflammation (innate immunity), which is mediated through the activation of the immune system. This type of inflammation persists only for a short time and is usually beneficial for the host. If the inflammation lasts for a longer period of time, the second stage of inflammation, or chronic inflammation, sets in and may predispose the host to various chronic illnesses, including cancer [68]. During inflammation, mast cells and leukocytes are recruited to the site of damage, which leads to a 'respiratory burst' due to an increased uptake of oxygen, and thus, an increased release and accumulation of ROS at the site of damage [7,65].

On the other hand, inflammatory cells also produce soluble mediators, such as metabolites of arachidonic acid, cytokines and chemokines, which act by further recruiting inflammatory cells to the site of damage and producing more reactive species. These key mediators can activate signal transduction cascades as well as induce changes in transcription factors, such as nuclear factor kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3), hypoxia-inducible factor-1α (HIF1-α), activator protein-1 (AP-1), nuclear factor of activated T cells (NFAT) and NF-E2 related factor-2 (Nrf2), which mediate immediate cellular stress responses (Figure 2). Induction of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), aberrant expression of inflammatory cytokines [tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6 and chemokines [IL-8; CXC chemokine receptor 4 (CXCR4)], as well as alterations in the expression of specific microRNAs, have also been reported to play a role in oxidative stress-induced inflammation [69]. This sustained inflammatory/oxidative environment leads to a vicious circle, which can damage healthy neighboring epithelial and stromal cells and over a long period of time may lead to carcinogenesis [70].

As an example, mutations in the rat sarcoma viral oncogene (RAS) induce an inflammatory response. RAS, which is mutated in approximately 25% of all malignancies [71], promotes cell proliferation, tumor growth, and angiogenesis of malignant cells. During inflammatory stimuli, Ras induces the expression of various inflammatory gene products, including the pro-inflammatory cytokines IL-1, IL-6 and IL-11 and the chemokine IL-8 [72].

3. Pro-oxidant network

Following an inflammatory stimulus, initiation of carcinogenesis mediated by ROS may be direct (oxidation, nitration, halogenation of nuclear DNA, RNA, and lipids), or mediated by the signaling pathways activated by ROS. With the help of the mitochondrial respiratory chain, aerobic organisms are able to attain a far greater energy production efficiency compared with anaerobic organisms. However, one disadvantage of aerobic respiration is continuous electron leakage to O_2 during mitochondrial ATP synthesis. In fact, 1–5% of total oxygen consumed in aerobic metabolism gives rise to the superoxide anion (O_2^-), an example of ROS. To protect against this free radical, the main enzyme for its degradation, the manganese-superoxide dismutase (Mn-SOD), dismutates it into H_2O_2 and water [73].

H_2O_2, another example of ROS, may be formed either by dismutation from superoxide anion or spontaneously in peroxisomes from molecular oxygen [74-76]. Despite its lesser reactivity compared with other ROS, H_2O_2 plays however an important role in carcinogenesis because it is capable of diffusing throughout the mitochondria and across cell

membranes and producing many types of cellular injury [74,75]. The main injurious effects of ROS in mammalian cells are however mediated by the hydroxyl radical (·OH). It has a very unstable electron structure and is therefore unable to diffuse more than one or two molecular diameters before it reacts in practice with any cellular component [76,77]. The majority of ·OH in vivo is produced in the presence of reduced transition metals (ions of Fe, Cu, Co, or Ni), mainly via the Fenton reaction when Fe^{2+} contacts H_2O_2. The ·OH-derived DNA damage includes the generation of 8-hydroxyguanosine (8-OHG), the hydrolysis product of which is 8-hydroxydeoxyguanosine (8-OHdG). 8-OHdG is the most widely used fingerprint of radical attack towards DNA [77,78]. 8-OHdG has been strongly implicated in carcinogenesis progression. For example, in breast carcinomas, 8-OHdG has been reported to be increased 8- to 17-fold in breast primary tumors compared with nonmalignant breast tissue [79-81].

$NO^{\cdot}$, another free radical implicated in carcinogenesis, is a short-lived free radical generated from L-arginine [82], that is effective against pathogens. The major part of $NO^{\cdot}$ is synthesized by iNOS, usually after challenge by immunological or inflammatory stimuli [82,83]. NO is synthesized from -arginine by the enzyme nitric oxide synthase (NOS). The constitutive (calcium-dependent) isoforms, neuronal NOS (nNOS or bNOS) and endothelial NOS (eNOS), produce small amounts of NO which act as a neurotransmittor and vasodilator, respectively [84]. The inducible (calcium-independent) isoform (iNOS) produces much larger amounts of NO and is only expressed during inflammation. Whereas iNOS can produce injurious amounts of RNS (check), eNOS and nNOS produce beneficial amounts under physiological conditions [85]. iNOS is induced by cytokines such as γ-interferon (γ-IFN), TNF-α, IL-1, and lipopolysaccharide (LPS). LPS activation induces the translocatation of NF-κB, from the cytoplasm to the nucleus, where it interacts with κB elements in the *NOS2 (iNOS)* 5′ flanking region, triggering *NOS2* transcription [86].

Defective autophagy of old mitochondria (mitophagy) can also be a major source of ROS [87]. These ROS produced by damaged mitochondria, can promote tumor development, likely by perturbing the signal transduction adaptor function of p62-controlling pathways [88].

To control the balance between production and removal of ROS (Figure 3), a variety of DNA repair enzymes exist, although antioxidants are more specific and efficient in protecting cells from radicals. This antioxidant system includes both endogenous and exogenous and enzymatic and non-enzymatic antioxidants. Glutathione (GSH), is a tripeptide and the major endogenous antioxidant produced by the cells, which helps to protect cells from ROS such as free radicals and peroxides [89]. It is now well established that ROS and electrophilic chemicals can damage DNA, and that GSH can protect against this type of damage [90]. GSH can also directly detoxify carcinogens through phase II metabolism and subsequent export of these chemicals from the cell. On the other hand, elevated GSH levels are observed in various types of cancerous cells and solid tumors, and this tends to make these cells and tissues more resistant to chemotherapy [91-93].

SODs were the first characterized antioxidant enzymes [94]. Three different types of SOD are expressed in human cells, copper-zinc SOD (Cu-ZnSOD), Mn-SOD, and extracellular-SOD (EC-SOD), all of which are able to dismutate two $O_2^{\cdot-}$ anions to H_2O_2 and molecular oxygen. Catalase is then responsible for detoxification of H_2O_2 to water. GPx are another group of enzymes capable of reducing hydroperoxides, including lipid hydroperoxides, using GSH as substrate. The oxidized form of glutathione disulfide (GSSG) is again reduced by the specific enzyme glutathione reductase. Peroxiredoxins (Prx) were first described 20 years ago and as in catalase and GPx, the main function of peroxiredoxins is to reduce alkyl hydroperoxides and H_2O_2 to the corresponding alcohol or water.

Direct effects of ROS, generally attributed to high concentrations at the site of damage, include DNA strand breaks, point mutations, aberrant DNA cross-linking, and mutations in proto-oncogenes and tumor-suppressor genes, thus promoting neoplastic transformation [7,95]. For example, ROS can reduce the expression and enzymatic activity of the DNA mismatch repair genes mutS homologue 2 and 6 and can increase the expression of DNA methyltransferases, leading to a global hypermethylation of the genome [60]. This leads to promoter silencing of several genes, such as adenomatous polyposis coli (APC), cyclin-dependent kinase inhibitor-2 (CDKN-2), breast cancer susceptibility gene 1 (BRCA1), retinoblastoma protein (Rb), and murine double minute 2 (MDM2), and the DNA mismatch repair gene, human mutL homolog 1 (hMLH1) [96,97].

On the other hand, low or transient levels of ROS can activate cellular proliferation or survival signaling pathways, such as the NF-κB, AP1, extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK), and phosphoinositide 3- kinase/AKT8 virus oncogene cellular homolog (PI3K/Akt) pathways (Table 3).

For example, H_2O_2 is able to degrade IκBα, the inhibitory subunit of NF-κB [137]. Protein kinase C, which participates in a variety of pathways regulating transcription and cell cycle control, is also activated by H_2O_2 [137]. In addition, ROS induces both the activation and synthesis of AP-1, a regulator of cell growth, proliferation, and apoptosis [138,139] and transcription factors such as STAT3, HIF-1α, and p53 [118,140,141].

4a. Cellular transformation

Chronic inflammation has been linked to various steps involved in carcinogenesis, including cellular transformation, promotion, survival, proliferation, invasion, angiogenesis, and metastasis [65,142]. How oxidative stress is involved in these various steps is discussed in the following sections.

Cancer is a multistage process defined by at least three stages: initiation, promotion, and progression [143-145]. Oxidative stress interacts with all three stages of this process. During the initiation stage, ROS may produce DNA damage by introducing gene mutations and structural alterations of the DNA. In the promotion stage, ROS can contribute to abnormal gene expression, blockage of cell- to cell communication, and modification of second messenger systems, thus resulting in an increase of cell proliferation or a decrease in apoptosis of the initiated cell population. Finally, oxidative stress may also participate in the progression stage of the cancer process by adding further DNA alterations to the initiated cell population [146].

In recent years, considerable evidence has demonstrated that ROS are involved in the link between chronic inflammation and cancer [147-149]. Indeed, an important characteristic of tumor promoters is their ability to recruit inflammatory cells and to stimulate them to generate ROS [150,151]. Tumor promotion, for example, can be inhibited in animal models by the use of agents, including certain antioxidants as well as steroids and retinoids, that can inhibit the phagocyte respiratory burst [148,150]. Moreover, increased levels of oxidatively modified DNA bases (such as thymidine glycol, 5-hydroxymethyl-2′-deoxyuridine and 8-OHdG) have been induced in the skin of mice by topical phorbol 12-myristate 13- acetate (PMA) exposure [152]. 8-OHdG has also been identified in the epidermis of nude mice exposed to near-UV [153]. In addition, genetic damage and neoplastic transformation have been demonstrated in cells co-cultured in vitro with activated phagocytes [149] and the genotoxic effects observed include formation of DNA strand breaks [151], sister chromatid exchange [154] and mutations [155]. Furthermore, the DNA base modifications observed are characteristic of an attack by reactive oxygen species OH. [156]. Inflammatory cells may also increase DNA damage by activating procarcinogens to DNA-damaging species, for

example neutrophils can activate aromatic amines, aflatoxins, estrogens, phenols, and polycyclic aromatic hydrocarbons by ROS-dependent mechanisms [148,157]. On the other hand, both neutrophils and macrophages have themselves been shown to release large quantities of superoxide, hydrogen peroxide, and hydroxyl radical following activation of their redox metabolism [158].

In fact, initial experiments on the role of ROS in tumor initiation have assumed that oxidative stress acts as a DNA-damaging agent, effectively increasing the mutation rate within cells and thus promoting oncogenic transformation [159]. However, more recent studies have revealed that in addition to inducing genomic instability, ROS can specifically activate certain signaling pathways and thus contribute to tumor development through the regulation of cellular proliferation, angiogenesis, and metastasis [160]. For example, nitrosative stress has been shown to play a critical role in inflammation-associated carcinogenesis by activating AP-1, a representative redox-sensitive transcription factor [161], which is involved in cell transformation and proliferation [139,162].

4b. Tumor cell survival

One of the key characteristics of tumor cells is their increased ability to survive compared with normal cells. ROS are reported to be tumorigenic by virtue of their ability to increase cell proliferation, survival, and cellular migration. ROS can induce DNA damage, leading to genetic lesions that initiate tumorigenicity and subsequent tumor progression. On the other hand, ROS can also induce cellular senescence and cell death and can therefore function as anti-tumorigenic agents. Whether ROS promote tumor cell survival or act as anti-tumorigenic agents depends on the cell and tissues, the location of ROS production, and the concentration of individual ROS.

ROS has been reported to play a major role in tumor initiation and survival induced by a variety of agents both in animal models and humans [158,163,164] by mediating cellular signal transduction pathways. These signaling pathways are involved in the transmission of inter or intracellular information and are critical for supporting tumor cell survival and establishing cell fate. The reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox) family of enzymes, one of the potential sources of ROS production, has been reported to promote tumor cell survival and growth [165]. For example, Nox4 and Nox5 promote tumor cell survival in pancreatic and lung cancers, respectively [165]. The serine-threonine kinase Akt has been reported to down-regulate antioxidant defenses and promote tumor cell survival [166]. ROS has also been reported to activate Akt by inhibiting phosphatase and tensin homolog deleted from chromosome 10 (PTEN), the phosphatase counteracting PI3K-dependent Akt activation [167]. Akt may foster tumorigenesis by multiple means [168,169], for example, by stabilizing cellular avian myeloblastosis virus oncogene (c-Myc) and cyclin D1 or by inducing degradation of the cyclin-dependent kinase (Cdk) inhibitor, p27 kinase inhibitor protein (p27Kip1). Akt is also a profound inhibitor of apoptosis due to its ability to inactivate pro-apoptotic molecules, including caspase-9 and the Bcl-2 homology3 (BH3)-only protein Bcl-XL/Bcl-2-associated death promoter (Bad), and by triggering the activity of the transcription factor NF-κB. In addition, Akt promotes nuclear translocation of the ubiquitin ligase MDM2, which counteracts p53-mediated apoptosis. An important aspect of Akt's promotion of cell survival involves alterations in cellular energy metabolism [168,169]. Thus, by preventing apoptosis and increasing oxidative metabolism, Akt lies at the hub of complex signaling networks that integrate a multitude of potentially oncogenic signals.

4c. Tumor cell proliferation

Uncontrolled tumor cell proliferation requires the upregulation of multiple intracellular signaling pathways including cascades involved in survival, proliferation, and cell cycle progression. The most significant effects of oxidants on signaling pathways have been observed in the mitogen-activated protein (MAP) kinase/AP-1 and NF-κB pathways [170]. The induction of redox-sensitive pathways during tumor cell proliferation is necessary since cell division presents tremendous energy requirements and the production of metabolites from energy-generating reactions must be buffered to prevent oxidative damage and ultimately cell death [171].

Of the MAP kinase family, which modulates gene expression through phosphorylation of a wide array of transcription factors, the ERK pathway is the most commonly linked with the regulation of cell proliferation. Activation of the ERK, c-Jun N-terminal kinase (JNK), and p38 subfamilies has been observed in response to changes in the cellular redox balance [172]. The induction of AP-1 by H_2O_2, cytokines, and other stressors, for example, is mediated mainly by JNK and p38 MAP kinase cascades [173]. Once activated, JNK proteins translocate to the nucleus and phosphorylate c-Jun and activating transcription factor-2 (ATF-2), enhancing transcriptional activities [174,175]. H_2O_2 can activate MAP kinases and thereby AP-1 in several manners.

Redox status has also been shown to have an impact on NF-κB regulation. NF-κB regulates several genes involved in cell transformation, proliferation, and angiogenesis [176]. Carcinogens and tumor promoters including UV radiation, phorbol esters, asbestos, alcohol, and benzo(a)pyrene are among the external stimuli that activate NF-κB [177,178]. Expression of NF-κB has been shown to promote cell proliferation, whereas inhibition of NF-κB activation blocks cell proliferation [179]. Additionally, tumor cells from blood neoplasms, and cell lines from different cancers, including colon, breast, pancreas, and squamous cell carcinoma, have all been reported to constitutively express activated NF-κB [180]. The mechanism for activation of NF-κB by ROS is not clear, and the relationship between NF-κB and ROS is complex [123]. Although mild oxidative stress can lead to modest NF-κB activation, extensive oxidative stress can inhibit NF-κB [123]. Furthermore, NF-κB can protect cells from oxidative stress through induction of the ferritin heavy chain and SOD2 genes, which are both regulated by NF-κB [181,182]. On the other hand, ROS are believed to be implicated as second messengers involved in activation of NF-κB via TNF and IL-1 [183] and indeed, suppression of TNF and IL-1 were shown to downregulate the expression of active NF-κB and inhibit proliferation of lymphoma and myelogenous leukemia cells [184]. The importance of ROS on NF-κB activation is further supported by studies demonstrating that activation of NF-κB by nearly all stimuli can be blocked by antioxidants, such as L-cysteine, N-acetylcysteine (NAC), thiols, green tea polyphenols, and vitamin E [185,186], although this might be not very specific because antioxidants have multiple targets [187]. Likewise, NF-κB activity was increased in cells that overexpressed SOD and decreased in cells overexpressing catalase [188].

Kinases, such as protein kinase C (PKC) can also be activated by H_2O_2 and redox cycling quinones [189,190]. Similarly, H_2O_2 leads to the activation of protein kinase B/Akt (PKB/Akt), which is associated with heat shock protein 27 (Hsp27) [191].

That ROS such as H_2O_2 and superoxide anion induce mitogenesis and cell proliferation has now been demonstrated in several mammalian cell types [192]; and a reduction in cellular oxidants via supplementation with antioxidants such as superoxide dismutase, catalase, β-carotene, and flavonoids inhibits cell proliferation in vitro [193]. However, paradoxically high concentrations of ROS can trigger apoptotic or necrotic cell death [194-196].

4d. Tumor cell invasion

Oxygen radicals may augment tumor invasion and metastasis by increasing the rates of cell migration. During transformation into invasive carcinoma, epithelial cells undergo profound alterations in morphology and adhesive mode, resulting in a loss of normal epithelial polarization and differentiation, and a switch to a more motile, invasive phenotype. For example, treatment of mammalian carcinoma cells with hydrogen peroxide prior to intravenous injection into mice enhances lung metastasis formation, indicating that an important function for ROS is the seeding of metastatic tumor cells [197]. This might be due to a decreased attachment of tumor cells to the basal lamina, or alternatively be due to the increased activity or expression of proteins that regulate cellular motility. For instance, oxidative stress regulates the expression of intercellular adhesion protein-1 (ICAM-1), a cell surface protein in endothelial and epithelial cells, most likely due to the activation of NF-κB. ICAM-1 together with IL-8 regulates the transendothelial migration of neutrophils and has a potential function in tumor metastasis [198].

On the other hand, it is believed that the matrix metalloproteinases (MMPs) play the central role, and their increased expression reportedly is associated with the invasion and metastasis of malignant tumors of different histogenetic origins [199]. For example, Mori et al. found that MMP-13, MMP-3, and MMP-10 were remarkably upregulated by the oxidant directly, and their activities were critically implicated in the invasive potential induced in NMuMG cells in the reconstituted model [200]. Another subgroup of MMPs, gelatinases (MMP-2 and -9), which are key enzymes for degrading type IV collagen and are thought to play a critical role in tumor invasion and metastasis [199], were also found to be activated post-transcriptionally by prolonged oxidative treatment. These effector molecules activated under prolonged oxidative stress relate chronic inflammation to malignant transformation, in particular to the invasive potential of cells, at least at a molecular level.

MMPs are capable of cleaving most components of the basement membrane and extracellular matrix [201]. The activation of MMPs, such as MMP-2, probably occurs by the reaction of ROS with thiol groups in the protease catalytic domain [202]. In additional to their role as key regulators of MMP activation, ROS have been implicated in MMP gene expression [203]. Both hydrogen peroxide and nitric oxide donors, as well as the increased expression of iNOS, stimulate the expression of several MMPs (MMP-1, MMP-3, MMP-9, MMP-10, MMP-13) [203]. In fibroblastic cells, the sustained production of H_2O_2 recently was shown to activate MMP-2 and to increase cell invasion [204]. Oxidative stress may also modulate MMP expression by activation of the rat sarcoma viral oncogene (RAS), or direct activation of the MAPK family members extracellular-signal regulated kinase 1/2 (ERK1/2), p38, and JNK, or inactivation of phosphatases that regulate these proteins [160].

In addition, several studies have reported the involvement of chemokines and chemokine receptors in the invasion and metastasis of different types of tumors [205-208]. The metastatic potential of chemokines is attributed to their ability to induce the expression of MMPs, which facilitate tumor invasion [208,209]. Moreover, silencing of endogenous CXCR4 gene expression by CXCR4-shRNA inhibited the proliferation, adhesion, chemotaxis and invasion of mucoepidermoid carcinoma cells [210]. In addition, recent data point to a role for the small guanosine triphosphatase Rac1 (GTPase Rac1) in motility and invasion of tumor cells in vitro by altering cell-cell and cell-matrix adhesion. For example, Rac1 activity induces ROS production in endothelial cells. These ROS can mediate Rac1-induced loss of cell-cell adhesion in primary human endothelial cells and thus might loosen the integrity of the endothelium [211].

It is becoming clear that a number of steps in the metastatic cascade, such as invasion, intravasation and extravasation are regulated by redox signaling [212]. One such redox

signalling molecule is the electrophilic cyclopentenone prostaglandin 15d-PGJ2 (15-deoxy-12,14 -prostaglandin J2), an inflammatory molecule [213], that can affect redox signalling through the post-translational modification of critical cysteine residues in proteins, such as actin, vimentin and tubulin [214,215]. The fact that 15d-PGJ2 can alter the cytoskeleton [212], may coincides with decreased migration and increased focal-adhesion disassembly, that might have important implications in the inhibition of metastatic processes such as invasion, intravasation and extravasation. These results suggest a role for redox signalling pathways, rather than direct cytoskeletal disruption, in the mechanism of 15d-PGJ2 in cancer cells.

Finally, Cheng et al demonstrated that ROS enhance the transendothelial migration (TEM) of melanoma cells during intravasation, and that this mechanism could potentially be triggered by ultraviolet radiation through the increased expression of thioredoxin interacting protein (Txnip) and inhibition of thioredoxin (Trx) [216].

4e. Tumor cell angiogenesis

Solid tumors induce an angiogenic response by the host blood vessels to form a new vascular network for the supply of nutrients and oxygen [217]. This neovascular response is partly responsible for tumor growth and metastatic spread [218,219]. Angiogenesis in tumors is controlled by the so-called ‘angiogenic switch,’ which allows the transition from low invasive and poorly vascularized tumors to highly invasive and angiogenic tumors. To further increase in size, tumor cells express a set of molecules that initiate tumor vascularization.

A number of cellular stress factors, including hypoxia, nutrient deprivation, and ROS, are important stimuli of angiogenic signaling [220]. In addition, overexpression of Ras has been linked to vascularization of tumors [221]. Indeed, transformation by Ras stabilizes HIF-1α and upregulates the transcription of vascular endothelial growth factor-A (VEGF-A). Moreover, chemical antioxidants inhibit the mitogenic activity of Ras, indicating that ROS participate directly in malignant transformation. Finally, ROS stabilize HIF-1α protein and induce production of angiogenic factors by tumor cells [222].

The HIF system plays a significant role in angiogenesis, and the molecular mechanisms of its regulation have recently been characterized. In addition, HIF-independent mechanisms that involve a number of other molecules and transcription factors such as NF-κB and p53 have been described. p53 may interact with the HIF system but may also have direct effects on angiogenesis regulators or interfere with translation mechanisms of angiogenesis factors

One other major factor in angiogenesis is vascular endothelial growth factor (VEGF), which is produced by the cells to stimulate the growth of new blood vessels. VEGF induces angiogenesis by stimulating endothelial cell proliferation and migration primarily through the receptor tyrosine kinase VEGF receptor2, fetal liver kinase 1/ kinase insert domain receptor (Flk1/KDR). VEGF binding initiates tyrosine phosphorylation of KDR, which results in activation of downstream signaling enzymes including ERK1/2, Akt and endothelial nitric oxide synthase (eNOS), which contribute to angiogenic-related responses in endothelial cells [134]. A number of oncogenes and tumor-suppressor genes that are normally associated with cell transformation [(RAS, c-Myc, murine sarcoma 3611 oncogene (RAF), human epidermal growth factor receptor-2 (HER-2/neu), c-Jun, and steroid receptor coactivator (SRC)] regulate angiogenesis through upregulation of VEGF or downregulation of thrombospondin-1 (TSP-1), an angiogenesis suppressor [223,224]. Furthermore, mutated p53 upregulates VEGF and in contrast, wild-type p53 decreases VEGF production and increases TSP-1 [225]. Angiogenic factors such as VEGF, fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) are released into the tumor microenvironment by

tumor or inflammatory cells in response to various stimuli, such as ROS [226]. The released growth factors activate endothelial cells that give rise to new blood vessels [227,228].

Monte et al. have demonstrated that lymphocyte-induced angiogenesis is triggered by ROS stimulation, and that this response can be blocked by the administration of a free radical scavenger to tumor bearing mice [229] [230]. In addition, the administration of H_2O_2 or an oxidative stress-producing drug (doxorubicin) to normal mice activated in vivo angiogenesis [229].

Due to reduced physiological tissue oxygen tension (hypoxia), which occurs during tumor initiation, tumors often become hypoxic. Under hypoxic conditions, cells activate signaling pathways, which regulate proliferation, angiogenesis, and death. Cancer cells have adapted to these pathways, effectively allowing tumors to survive and even grow under adverse hypoxic conditions [160]. This adaptation of tumor cells to hypoxia contributes to the malignant phenotype and to aggressive tumor progression [231], and low oxygen tension in tumors is associated with increased metastasis and poor survival of patients with several forms of squamous tumor [232,233]. HIF-1α responds to these changes by specifically decreasing the oxygen (or hypoxia) level, and upregulating several genes to promote survival in low-oxygen conditions and thus promoting angiogenesis.

In conclusion, although previous sections indicate that all different sub-stages of tumor development are affected by ROS and inflammation, early stages of cancer development (e.g. cellular transformation), involving DNA damage, are however most affected by ROS generated inflammation. For example, colitis may develop into colon cancer after inflammatory infiltration, increased production of ROS, impairment of antioxidant defenses, DNA damage, and genetic and epigenetic alterations, resulting in the transformation of epithelial cells [234]. Or, bronchitis, which can lead to lung cancer, clearly links pro-oxidants, generated by cigarette smoke, to inflammation of the bronchus, and eventually transformation of lung cells into lung cancer [235]. Similarly pancreatitis and esophagitis, both induced by tobacco and alcohol, may transform normal tissue into pancreatic or esophageal cancer if the antioxidant system is not sufficiently effective [236,237].

4f. Chemoresistance

Despite many decades of research, the mechanisms underlying chemoresistance are still poorly understood. There is growing evidence that the inflammatory tumor microenvironment modulates not only cancer development but also cancer responsiveness and resistance to conventional anticancer therapies [238]. Experimental studies have led to the identification of various cancer cell-intrinsic resistance mechanisms, e.g., activation and/or overexpression of drug transporter proteins (e.g., P-glycoprotein), altered expression of detoxifying enzymes (e.g., glutathione S-transferase) or resistance to apoptosis/senescence pathways [239-242].

For example, an inflammatory response induces changes in expression and activity of multidrug-resistance (MDR)-associated protein transporters, greatly affecting drug responses [243,244]. It has been shown that acute inflammation suppresses the drug transporter P-glycoprotein (PGP) in the liver, whereas it activates PGP in kidneys, resulting in changes in the pharmacokinetics of the PGP substrate doxorubicin [245]. Likewise, expression of multidrug resistance-associated protein 1 (MRP1) is elevated in inflamed intestine of patients with Crohn's disease or ulcerative colitis [246]. Thus, enhanced states of inflammation influence proteins that are strongly linked with drug resistance.

In addition to the effects caused by inflammation, several chemotherapeutic agents have also been shown to activate the transcription factor NF-κB in human lung and cervical cancers

and in T cells [247-249]. These agents are paclitaxel, vinblastine, vincristine, doxorubicin, daunomycin, 5-fluorouracil, cisplatin, and tamoxifen. Activation of NF-κB by these agents has been linked in turn with chemoresistance through serine phosphorylation of inhibitor of κBα (IκBα) [250,251]. Various in vitro studies have supported a link between NF-κB activation, cytokine production and chemoresistance. One pathway via which NF-κB can be activated is the Toll-like receptor (TLR) pathway. TLRs generally signal via the adapter protein myeloid differentiation primary response gene 88 (MyD88) leading to activation of NF-κB and production of pro-inflammatory cytokines. Activation of TLR signaling in ovarian cancer cell lines by exogenously added LPS resulted in an activated NF-κB pathway, which promoted secretion of proinflammatory cytokines and subsequently conferred resistance to paclitaxel [252,253]. Also, TNF receptor signaling promotes NF-κB activation and has been linked with chemoresistance. For example, exposure of breast cancer cells to exogenously added TNFα results in selection for breast cancer cells that overexpress NF-κB, leading to increased cancer cell survival and resistance to ionizing radiation [254]. At the same time, cytokines produced by stromal cells in the tumor microenvironment (e.g., IL-1 or TNFα) could potentially activate the NF-κB pathway in cancer cells and thus contribute to chemoresistance. These data call for functional in vivo studies to elucidate the involvement of the inflammatory tumor microenvironment in NF-κB-dependent chemoresistance.

Another mechanism that might be involved in chemoresistance is increased levels of GSH in cancer cells [92]. In particular, the overexpression of glutathione S-transferases (GST), the enzymes that catalyse the conjugation of reduced glutathione to electrophilic [255], as well as efflux pumps, may reduce the reactivity of various anticancer drugs [256]. The increase of the GST levels occurs by transcriptional activation mediated by the nuclear factor-erythroid 2 p45-related factor 2 (Nrf2) [257]. Indeed, using genetic manipulation, Lau et al. have demonstrated a strong positive correlation between Nrf2 levels and resistance of three cancer cell lines to chemotherapeutic drugs such as cisplatin, doxorubicin, and etoposide [258]. Chemical activation of Nrf2 by pretreatment with tertiary-butylhydroquinone (tBHQ) also increased survival of neuroblastoma cells in response to the three drugs tested [259]. Consistent with these findings, the role of Nrf2 in determining efficacy of cisplatin was also demonstrated in ovarian cancer cells using siRNA knockdown of Nrf2 [260]. Moreover, many kelch-like ECH-associated protein 1 (Keap1) mutations or loss of heterozygosity in the Keap1 locus have been identified in lung cancer cell lines or cancer tissues [261,262]. Keap1 mutations or loss of heterozygosity resulted in inactivation of Keap1 or a reduced expression of Keap1, which upregulated the protein level of Nrf2 and transactivation of its downstream genes [261,262]. Similar to Nrf2, the protective effect of heme oxygenase-1 (HMOX-1, or HO-1) in normal cells may protect from oxidative stress-related diseases. However, such an effect is undesirable in cancer because it provides a selective advantage for cancer cells to survive. Consistent with this notion, HMOX-1 has been found to be overexpressed in various tumor types. It is believed that overexpression of HMOX-1 facilitates cancer cell growth and survival in many ways, such as stimulating rapid growth of cancer cells, enhancing cancer cell resistance to stress and apoptosis, promoting angiogenesis of tumors, and aiding in metastasis of tumors [263]. In addition to HMOX-1, other Nrf2-downstream genes such as Prx1, GPx, and thioredoxin reductase (TrxR) were also upregulated in many cancer cells or tissues and may contribute to chemoresistance [264-266]. In ovarian cancer, constitutive activation of ERK activity has been associated with high tumorigenicity and chemoresistance [267,268]. In addition, functional analyses employing knockdown of MKP3, a member of the subfamily of protein tyrosine phosphatases known as dual-specificity phosphatases (MKPs) [269,270], and ectopic overexpression revealed the role of MKP3 in negatively regulating ERK1/2 activity and inhibiting tumorigenicity and chemoresistance in vitro and in vivo. MKP3 is capable of

dephosphorylating ERK1/2 by protein-protein interactions via mitogen-activated protein kinase interaction motif within the N-terminal ERK1/2-binding domain [271].

4g. Radioresistance

Acquired tumor radioresistance can be induced during radiotherapy owing to tumor repopulation [272]. Although tumor radioresistance stands as a fundamental barrier limiting the effectiveness of radiation therapy, the exact molecular mechanisms underlying the radioadaptive response are largely unknown (Figure 4). Olivieri et al. [273] first described an adaptive response of human lymphocytes to ionizing radiation. Since then, a substantial number of reports have made a strong case for the existence of cellular radioprotective mechanisms that can be activated in response to a small dose of ionizing radiation. It is assumed that a specific pro-survival signaling network is induced in irradiated mammalian cells.

The elevated basal NF-κB activity in certain cancers has been linked with tumor resistance to chemotherapy and radiation [274]. NF-κB in adaptive radioresistance is evidenced in mouse epidermal cells [275] and human keratinocytes, and inhibition of NF-κB blocks the adaptive radioresistance [275]. Human breast cancer cells treated with fractional γ-irradiation show an enhanced clonogenic survival and NF-κB activation [276,277]. Blocking NF-κB inhibited the adaptive radioresistance. These results provide the first evidence that activation of NF-κB is required for signaling the radio-adaptive resistance by exposure to radiation. Together with the assumption that NF-κB is able to regulate more than 150 effector genes, these results suggest that NF-κB plays a key role in tumor radioadaptive resistance under fractional ionizing radiation. Furthermore, in a study [278] that immunocytochemically examined the levels of activated NF-κB protein in pretreatment cancer specimens and in resected specimens of patients with chemoradiotherapy resistance, the cancers expressed higher levels of cytoplasmic NF-κB than did the adjacent nonmalignant mucosa. Furthermore, Sandur et al. suggest that transient inducible NF-κB activation provides a prosurvival response to radiation that may account for the development of radioresistance [279].

On the other hand, hypoxia is a principal signature of the tumor microenvironment and is considered to be the most important cause of clinical radioresistance and local treatment failure. The response of cells to ionizing radiation is strongly dependent upon oxygen, which is traditionally explained by the “oxygen fixation hypothesis” [280]. Oxygen is so far the best radiosensitizer. De Ridder et al. demonstrated that iNOS, activated by pro-inflammatory cytokines, can radiosensitize tumor cells through endogenous production of NO [280]. They further observed that this radiosensitizing effect is transcriptionally controlled by hypoxia and by NF-κB. Consistently, NF-κB inhibition has been used as an approach to radiosensitize tumor cells, aiming at stimulating apoptosis and inhibiting DNA repair. Moreover, the inflammatory mediators TNFα and NO have been repeatedly used as targets to radiosensitize tumor cells [281-285].

4h. Stem cell survival

Cancer stem cells (CSCs) are cancer cells that have the ability to generate tumors through the processes of self-renewal and differentiation into multiple cells. Such cells persist in tumors as a distinct population and cause relapse and metastasis by giving rise to new tumors. The existence of CSCs may have several implications in cancer treatment, including disease identification, selection of drug targets, prevention of metastasis, and development of new intervention strategies.

The first conclusive evidence for CSCs was published in 1997 [286], and to date CSCs have been isolated from both leukemias and a variety of solid tumors, including breast, brain, pancreatic, prostrate, ovary, and colon cancers [287-293]. The pathways that regulate self-renewal of CSCs include wint (Wnt), Notch, Hedgehog, and tumor-suppressor genes such as PTEN and TP53 (tumor protein 53) [294]. Although redox balance plays an important role in the maintenance of stem cell self-renewal and in differentiation, redox status in CSCs has yet to be explored. However, given the similarity between normal stem cells and CSCs and the fact that redox status plays an important role in cancer cell development, it is tempting to speculate that redox status may have a role in CSC survival. A recent study by Diehn et al. demonstrated that, similar to normal stem cells, subsets of CSCs in human and murine breast tumors have lower ROS levels than do the corresponding non-tumorigenic cells [295]. The group further showed that lower levels of ROS were associated with increased free radical scavenging systems and that pharmacologic depletion of these scavengers significantly decreased clonogenicity and resulted in radiosensitization of CSCs. Additionally, two studies showed that CD133+ CSCs conferred chemoresistance to cisplatin and doxorubicin (known ROS generators) in ovarian cancer cells [296] and hepatocellular carcinoma [297], respectively. These studies further indicate that redox status may be important in maintaining CSC survival.

4i. Stromal cell signaling

Cancer progression must involve both genetic and behavioral changes in cancer cells, and these changes are in part driven by the cancer-associated stromal cells and tumor microenvironment [298,299]. The stromal component of the normal prostate epithelium, for example, consists of smooth muscle, fibroblasts, vascular endothelial cells, nerve cells, inflammatory cells, insoluble matrix, and soluble factors [300]. Studies by De Marzo et al. highlight the role of inflammation in prostate cancer, suggesting that atrophic lesions are an early event in prostate carcinogenesis [301]. The macrophages in the tumor microenvironment produce ROS and RNS. The resulting increases in superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical, and free iron damage DNA, causing genetic mutations and initiating cancer progression. Tissue and cell recombination studies demonstrate the important regulatory role of fibromuscular stroma and stromal fibroblasts in prostate development and prostate carcinogenesis [300]. Cancer cells and stromal cells interact through physical contact or through soluble factors or insoluble extracellular matrix (ECM) factors. These stromal fibroblasts, which interact with cancer cells, have increased levels of brain-derived neurotropic factor, chemokines, CC chemokine ligand 5 (CCL5) and CXC chemokine lix 5 (CXCL5), versican, tenascin, connective tissue growth factor, stromal cell derived factor-1/ CXC chemokine ligand 12 (SDF-1/CXCL12), and HIF-1α [302]. Other studies have demonstrated the role of stromal soluble factors interacting with receptors on prostate cancer cells. The stromal factors include VEGF, bFGF, hepatocyte growth factor/ scatter factor (HGF/SF), transforming growth factor-β (TGF-β), insulin like growth factor-1 (IGF-1), IL-6, and keratinocyte growth factor (KGF) [303].

Several studies have found that tumors promote a constant influx of myelomonocytic cells that express inflammatory mediators supporting pro-tumoral functions. Myelomonocytic cells are key orchestrators of cancer-related inflammation associated with proliferation and survival of malignant cells, subversion of adaptive immune response, angiogenesis, stroma remodeling, and metastasis formation [304].

Tumor-derived factors, which cause sustained myelopoiesis, accumulation, and functional differentiation of myelomonocytic cells, provide an essential support for the angiogenesis and the stroma remodeling required for tumor growth [305,306]. In addition, it has long been known that tumor growth is promoted by tumor-associated macrophages (TAM), a major leukocyte population present in tumors [65,307-310]. Accordingly, in many but not

all human tumors, a high frequency of infiltrating TAM is associated with poor prognosis. A model by which macrophages promote tumor invasion and metastasis includes expression of their proteolytic activity and subsequent breakdown of the basement membrane around the preinvasive tumors, thereby enhancing the ability of tumor cells to escape into the surrounding stroma [311]. In lung cancer, for example, TAM may favor tumor progression by contributing to stroma formation and angiogenesis through their release of platelet-derived growth factor, in conjunction with TGF-β production by cancer cells [310]. TAM produce several MMPs, such as MMP-2 and MMP-9, that degrade proteins in the extracellular matrix and also produce activators of MMPs, such as chemokines.

5. Conclusion

This review clearly implicates the role of ROS in different phases of tumorigenesis. Therefore, targeting redox-sensitive pathways and transcription factors offers great promise for cancer prevention and therapy. Numerous agents have been identified that can interfere with redox cell signaling pathways [9,312,313]. These include neutraceuticals derived from fruits, vegetables, spices, grains, and cereals. They have been shown to suppress tumorigenesis in preclinical models. Whether these agents can inhibit tumor growth in patients remains to be elucidated.

Acknowledgments

We thank Michael Worley for carefully editing the manuscript. Dr. Aggarwal is the Ransom Horne, Jr., Professor of Cancer Research. This work was supported by a grant from the Clayton Foundation for Research (B.B.A.), a core grant from the National Institutes of Health (CA-16672), a program project grant from National Institutes of Health (NIH CA-124787-01A2), and a grant from the Center for Targeted Therapy of MD Anderson Cancer Center. Simone Reuter was supported by a grant from the Fonds National de la Recherche Luxembourg (PDR-08-017).

References

1. Durackova Z. Some current insights into oxidative stress. Physiol Res. 2009
2. Jabs T. Reactive oxygen intermediates as mediators of programmed cell death in plants and animals. Biochem Pharmacol 1999;57:231–245. [PubMed: 9890550]
3. Poyton RO, Ball KA, Castello PR. Mitochondrial generation of free radicals and hypoxic signaling. Trends Endocrinol Metab 2009;20:332–340. [PubMed: 19733481]
4. Fridovich I. The biology of oxygen radicals. Science 1978;201:875–880. [PubMed: 210504]
5. Goossens V, De Vos K, Vercammen D, Steemans M, Vancompernolle K, Fiers W, Vandenabeele P, Grooten J. Redox regulation of TNF signaling. Biofactors 1999;10:145–156. [PubMed: 10609876]
6. Goossens V, Grooten J, De Vos K, Fiers W. Direct evidence for tumor necrosis factor-induced mitochondrial reactive oxygen intermediates and their involvement in cytotoxicity. Proc Natl Acad Sci U S A 1995;92:8115–8119. [PubMed: 7667254]
7. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. Nat Rev Cancer 2003;3:276–285. [PubMed: 12671666]
8. Schraufstatter I, Hyslop PA, Jackson JH, Cochrane CG. Oxidant-induced DNA damage of target cells. J Clin Invest 1988;82:1040–1050. [PubMed: 2843565]
9. Fang J, Seki T, Maeda H. Therapeutic strategies by modulating oxygen stress in cancer and inflammation. Adv Drug Deliv Rev 2009;61:290–302. [PubMed: 19249331]
10. Khandrika L, Kumar B, Koul S, Maroni P, Koul HK. Oxidative stress in prostate cancer. Cancer Lett 2009;282:125–136. [PubMed: 19185987]
11. Visconti R, Grieco D. New insights on oxidative stress in cancer. Curr Opin Drug Discov Devel 2009;12:240–245.
12. Briganti S, Picardo M. Antioxidant activity, lipid peroxidation and skin diseases. What's new. J Eur Acad Dermatol Venereol 2003;17:663–669. [PubMed: 14761133]

13. Kinnula VL, Crapo JD. Superoxide dismutases in the lung and human lung diseases. Am J Respir Crit Care Med 2003;167:1600–1619. [PubMed: 12796054]
14. Minelli A, Bellezza I, Conte C, Culig Z. Oxidative stress-related aging: A role for prostate cancer? Biochim Biophys Acta 2009;1795
15. Sies H. Oxidative stress: from basic research to clinical application. Am J Med 1991;91:31S–38S. [PubMed: 1928209]
16. Wilson JN, Pierce JD, Clancy RL. Reactive oxygen species in acute respiratory distress syndrome. Heart Lung 2001;30:370–375. [PubMed: 11604979]
17. Dugan LL, Quick KL. Reactive oxygen species and aging: evolving questions. Sci Aging Knowledge Environ 2005;2005:pe20. [PubMed: 15994214]
18. Hensley K, Butterfield DA, Hall N, Cole P, Subramaniam R, Mark R, Mattson MP, Markesbery WR, Harris ME, Aksenov M, et al. Reactive oxygen species as causal agents in the neurotoxicity of the Alzheimer's disease-associated amyloid beta peptide. Ann N Y Acad Sci 1996;786:120–134. [PubMed: 8687014]
19. Multhaup G, Ruppert T, Schlicksupp A, Hesse L, Beher D, Masters CL, Beyreuther K. Reactive oxygen species and Alzheimer's disease. Biochem Pharmacol 1997;54:533–539. [PubMed: 9337068]
20. Halliwell B. Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. Br J Exp Pathol 1989;70:737–757. [PubMed: 2557883]
21. Lau AT, Wang Y, Chiu JF. Reactive oxygen species: current knowledge and applications in cancer research and therapeutic. J Cell Biochem 2008;104:657–667. [PubMed: 18172854]
22. Renschler MF. The emerging role of reactive oxygen species in cancer therapy. Eur J Cancer 2004;40:1934–1940. [PubMed: 15315800]
23. Weinberg F, Chandel NS. Reactive oxygen species-dependent signaling regulates cancer. Cell Mol Life Sci 2009;66:3663–3673. [PubMed: 19629388]
24. Touyz RM. Reactive oxygen species and angiotensin II signaling in vascular cells -- implications in cardiovascular disease. Braz J Med Biol Res 2004;37:1263–1273. [PubMed: 15273829]
25. Yoshizumi M, Tsuchiya K, Tamaki T. Signal transduction of reactive oxygen species and mitogen-activated protein kinases in cardiovascular disease. J Med Invest 2001;48:11–24. [PubMed: 11286012]
26. Muhammad S, Bierhaus A, Schwaninger M. Reactive oxygen species in diabetes-induced vascular damage, stroke, and Alzheimer's disease. J Alzheimers Dis 2009;16:775–785. [PubMed: 19387112]
27. Di Virgilio F. New pathways for reactive oxygen species generation in inflammation and potential novel pharmacological targets. Curr Pharm Des 2004;10:1647–1652. [PubMed: 15134562]
28. Moulton PJ. Inflammatory joint disease: the role of cytokines, cyclooxygenases and reactive oxygen species. Br J Biomed Sci 1996;53:317–324. [PubMed: 9069110]
29. Bolanos JP, Moro MA, Lizasoain I, Almeida A. Mitochondria and reactive oxygen and nitrogen species in neurological disorders and stroke: Therapeutic implications. Adv Drug Deliv Rev 2009;61:1299–1315. [PubMed: 19716390]
30. Atabek ME, Vatansev H, Erkul I. Oxidative stress in childhood obesity. J Pediatr Endocrinol Metab 2004;17:1063–1068. [PubMed: 15379416]
31. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–1761. [PubMed: 15599400]
32. Tabner BJ, Turnbull S, El-Agnaf O, Allsop D. Production of reactive oxygen species from aggregating proteins implicated in Alzheimer's disease, Parkinson's disease and other neurodegenerative diseases. Curr Top Med Chem 2001;1:507–517. [PubMed: 11895127]
33. Tieu K, Ischiropoulos H, Przedborski S. Nitric oxide and reactive oxygen species in Parkinson's disease. IUBMB Life 2003;55:329–335. [PubMed: 12938735]
34. Kamp DW, Graceffa P, Pryor WA, Weitzman SA. The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 1992;12:293–315. [PubMed: 1577332]

35. Kinnula VL, Fattman CL, Tan RJ, Oury TD. Oxidative stress in pulmonary fibrosis: a possible role for redox modulatory therapy. Am J Respir Crit Care Med 2005;172:417–422. [PubMed: 15894605]

36. Gelderman KA, Hultqvist M, Olsson LM, Bauer K, Pizzolla A, Olofsson P, Holmdahl R. Rheumatoid arthritis: the role of reactive oxygen species in disease development and therapeutic strategies. Antioxid Redox Signal 2007;9:1541–1567. [PubMed: 17678439]

37. Haurani MJ, Pagano PJ. Adventitial fibroblast reactive oxygen species as autacrine and paracrine mediators of remodeling: bellwether for vascular disease? Cardiovasc Res 2007;75:679–689. [PubMed: 17689510]

38. Jeremy JY, Shukla N, Muzaffar S, Handley A, Angelini GD. Reactive oxygen species, vascular disease and cardiovascular surgery. Curr Vasc Pharmacol 2004;2:229–236. [PubMed: 15320821]

39. Miyajima A, Nakashima J, Yoshioka K, Tachibana M, Tazaki H, Murai M. Role of reactive oxygen species in cis-dichlorodiammineplatinum-induced cytotoxicity on bladder cancer cells. Br J Cancer 1997;76:206–210. [PubMed: 9231920]

40. Salganik RI, Albright CD, Rodgers J, Kim J, Zeisel SH, Sivashinskiy MS, Van Dyke TA. Dietary antioxidant depletion: enhancement of tumor apoptosis and inhibition of brain tumor growth in transgenic mice. Carcinogenesis 2000;21:909–914. [PubMed: 10783311]

41. Brown NS, Bicknell R. Hypoxia and oxidative stress in breast cancer. Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Res 2001;3:323–327. [PubMed: 11597322]

42. Sharma A, Rajappa M, Satyam A, Sharma M. Oxidant/anti-oxidant dynamics in patients with advanced cervical cancer: correlation with treatment response. Mol Cell Biochem 341:65–72. [PubMed: 20354762]

43. Oliveira CP, Kassab P, Lopasso FP, Souza HP, Janiszewski M, Laurindo FR, Iriya K, Laudanna AA. Protective effect of ascorbic acid in experimental gastric cancer: reduction of oxidative stress. World J Gastroenterol 2003;9:446–448. [PubMed: 12632494]

44. Calvisi DF, Ladu S, Hironaka K, Factor VM, Thorgeirsson SS. Vitamin E down-modulates iNOS and NADPH oxidase in c-Myc/TGF-alpha transgenic mouse model of liver cancer. J Hepatol 2004;41:815–822. [PubMed: 15519655]

45. Azad N, Rojanasakul Y, Vallyathan V. Inflammation and lung cancer: roles of reactive oxygen/nitrogen species. J Toxicol Environ Health B Crit Rev 2008;11:1–15. [PubMed: 18176884]

46. Fruehauf JP, Trapp V. Reactive oxygen species: an Achilles' heel of melanoma? Expert Rev Anticancer Ther 2008;8:1751–1757. [PubMed: 18983235]

47. Kuku I, Aydogdu I, Bayraktar N, Kaya E, Akyol O, Erkurt MA. Oxidant/antioxidant parameters and their relationship with medical treatment in multiple myeloma. Cell Biochem Funct 2005;23:47–50. [PubMed: 15386538]

48. Sumi D, Shinkai Y, Kumagai Y. Signal transduction pathways and transcription factors triggered by arsenic trioxide in leukemia cells. Toxicol Appl Pharmacol 244:385–392. [PubMed: 20193703]

49. van de Wetering CI, Coleman MC, Spitz DR, Smith BJ, Knudson CM. Manganese superoxide dismutase gene dosage affects chromosomal instability and tumor onset in a mouse model of T cell lymphoma. Free Radic Biol Med 2008;44:1677–1686. [PubMed: 18291119]

50. Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. Cancer 2007;109:54–59. [PubMed: 17099862]

51. Chan DW, Liu VW, Tsao GS, Yao KM, Furukawa T, Chan KK, Ngan HY. Loss of MKP3 mediated by oxidative stress enhances tumorigenicity and chemoresistance of ovarian cancer cells. Carcinogenesis 2008;29:1742–1750. [PubMed: 18632752]

52. Edderkaoui M, Hong P, Vaquero EC, Lee JK, Fischer L, Friess H, Buchler MW, Lerch MM, Pandol SJ, Gukovskaya AS. Extracellular matrix stimulates reactive oxygen species production and increases pancreatic cancer cell survival through 5-lipoxygenase and NADPH oxidase. Am J Physiol Gastrointest Liver Physiol 2005;289:G1137–1147. [PubMed: 16037546]

53. Ma Q, Cavallin LE, Yan B, Zhu S, Duran EM, Wang H, Hale LP, Dong C, Cesarman E, Mesri EA, Goldschmidt-Clermont PJ. Antitumorigenesis of antioxidants in a transgenic Rac1 model of Kaposi's sarcoma. Proc Natl Acad Sci U S A 2009;106:8683–8688. [PubMed: 19429708]

54. Bartsch H, Nair J. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbecks Arch Surg 2006;391:499–510. [PubMed: 16909291]
55. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell 140:883–899. [PubMed: 20303878]
56. Grivennikov SI, Karin M. Inflammation and oncogenesis: a vicious connection. Curr Opin Genet Dev 20:65–71. [PubMed: 20036794]
57. Gonda TA, Tu S, Wang TC. Chronic inflammation, the tumor microenvironment and carcinogenesis. Cell Cycle 2009;8:2005–2013. [PubMed: 19550141]
58. Garodia P, Ichikawa H, Malani N, Sethi G, Aggarwal BB. From ancient medicine to modern medicine: ayurvedic concepts of health and their role in inflammation and cancer. J Soc Integr Oncol 2007;5:25–37. [PubMed: 17309811]
59. Aggarwal BB, Gehlot P. Inflammation and cancer: how friendly is the relationship for cancer patients? Curr Opin Pharmacol 2009;9:351–369. [PubMed: 19665429]
60. Schetter AJ, Heegaard NH, Harris CC. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. Carcinogenesis 31:37–49. [PubMed: 19955394]
61. Ekbom A, Helmick C, Zack M, Adami HO. Increased risk of large-bowel cancer in Crohn's disease with colonic involvement. Lancet 1990;336:357–359. [PubMed: 1975343]
62. Ekbom A, Helmick C, Zack M, Adami HO. Ulcerative colitis and colorectal cancer. A population-based study. N Engl J Med 1990;323:1228–1233. [PubMed: 2215606]
63. Gillen CD, Walmsley RS, Prior P, Andrews HA, Allan RN. Ulcerative colitis and Crohn's disease: a comparison of the colorectal cancer risk in extensive colitis. Gut 1994;35:1590–1592. [PubMed: 7828978]
64. Ekbom A, McLaughlin JK, Nyren O. Pancreatitis and the risk of pancreatic cancer. N Engl J Med 1993;329:1502–1503. [PubMed: 8413467]
65. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–867. [PubMed: 12490959]
66. Kundu JK, Surh YJ. Inflammation: gearing the journey to cancer. Mutat Res 2008;659:15–30. [PubMed: 18485806]
67. Aggarwal BB, Vijayalekshmi RV, Sung B. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe. Clin Cancer Res 2009;15:425–430. [PubMed: 19147746]
68. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. J Clin Invest 2007;117:1175–1183. [PubMed: 17476347]
69. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 2007;121:2373–2380. [PubMed: 17893866]
70. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. Int J Cancer 2007;121:2381–2386. [PubMed: 17893868]
71. Bos JL. ras oncogenes in human cancer: a review. Cancer Res 1989;49:4682–4689. [PubMed: 2547513]
72. Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. Cancer Cell 2004;6:447–458. [PubMed: 15542429]
73. Karihtala P, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. APMIS 2007;115:81–103. [PubMed: 17295675]
74. Mates JM, Sanchez-Jimenez FM. Role of reactive oxygen species in apoptosis: implications for cancer therapy. Int J Biochem Cell Biol 2000;32:157–170. [PubMed: 10687951]
75. Ray G, Husain SA. Oxidants, antioxidants and carcinogenesis. Indian J Exp Biol 2002;40:1213–1232. [PubMed: 13677623]
76. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004;266:37–56. [PubMed: 15646026]
77. Marnett LJ. Oxyradicals and DNA damage. Carcinogenesis 2000;21:361–370. [PubMed: 10688856]

78. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 1996;313(Pt 1):17–29. [PubMed: 8546679]

79. Malins DC, Haimanot R. Major alterations in the nucleotide structure of DNA in cancer of the female breast. Cancer Res 1991;51:5430–5432. [PubMed: 1655250]

80. Matsui A, Ikeda T, Enomoto K, Hosoda K, Nakashima H, Omae K, Watanabe M, Hibi T, Kitajima M. Increased formation of oxidative DNA damage, 8-hydroxy-2′-deoxyguanosine, in human breast cancer tissue and its relationship to GSTP1 and COMT genotypes. Cancer Lett 2000;151:87–95. [PubMed: 10766427]

81. Musarrat J, Arezina-Wilson J, Wani AA. Prognostic and aetiological relevance of 8-hydroxyguanosine in human breast carcinogenesis. Eur J Cancer 1996;32A:1209–1214. [PubMed: 8758255]

82. Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. J Biol Chem 1994;269:13725–13728. [PubMed: 7514592]

83. Davis KL, Martin E, Turko IV, Murad F. Novel effects of nitric oxide. Annu Rev Pharmacol Toxicol 2001;41:203–236. [PubMed: 11264456]

84. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993;329:2002–2012. [PubMed: 7504210]

85. Lowenstein CJ, Padalko E. iNOS (NOS2) at a glance. J Cell Sci 2004;117:2865–2867. [PubMed: 15197240]

86. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. J Biol Chem 1994;269:4705–4708. [PubMed: 7508926]

87. Zhang Y, Qi H, Taylor R, Xu W, Liu LF, Jin S. The role of autophagy in mitochondria maintenance: characterization of mitochondrial functions in autophagy-deficient S. cerevisiae strains. Autophagy 2007;3:337–346. [PubMed: 17404498]

88. Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, Bray K, Reddy A, Bhanot G, Gelinas C, Dipaola RS, Karantza-Wadsworth V, White E. Autophagy suppresses tumorigenesis through elimination of p62. Cell 2009;137:1062–1075. [PubMed: 19524509]

89. Pompella A, Visvikis A, Paolicchi A, De Tata V, Casini AF. The changing faces of glutathione, a cellular protagonist. Biochem Pharmacol 2003;66:1499–1503. [PubMed: 14555227]

90. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44–84. [PubMed: 16978905]

91. Calvert P, Yao KS, Hamilton TC, O'Dwyer PJ. Clinical studies of reversal of drug resistance based on glutathione. Chem Biol Interact 1998;111-112:213–224. [PubMed: 9679556]

92. Balendiran GK, Dabur R, Fraser D. The role of glutathione in cancer. Cell Biochem Funct 2004;22:343–352. [PubMed: 15386533]

93. Estrela JM, Ortega A, Obrador E. Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci 2006;43:143–181. [PubMed: 16517421]

94. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049–6055. [PubMed: 5389100]

95. Meira LB, Bugni JM, Green SL, Lee CW, Pang B, Borenshtein D, Rickman BH, Rogers AB, Moroski-Erkul CA, McFaline JL, Schauer DB, Dedon PC, Fox JG, Samson LD. DNA damage induced by chronic inflammation contributes to colon carcinogenesis in mice. J Clin Invest 2008;118:2516–2525. [PubMed: 18521188]

96. Das PM, Singal R. DNA methylation and cancer. J Clin Oncol 2004;22:4632–4642. [PubMed: 15542813]

97. Fleisher AS, Esteller M, Harpaz N, Leytin A, Rashid A, Xu Y, Liang J, Stine OC, Yin J, Zou TT, Abraham JM, Kong D, Wilson KT, James SP, Herman JG, Meltzer SJ. Microsatellite instability in inflammatory bowel disease-associated neoplastic lesions is associated with hypermethylation and diminished expression of the DNA mismatch repair gene, hMLH1. Cancer Res 2000;60:4864–4868. [PubMed: 10987299]

98. Park JH, Mangal D, Frey AJ, Harvey RG, Blair IA, Penning TM. Aryl hydrocarbon receptor facilitates DNA strand breaks and 8-oxo-2′-deoxyguanosine formation by the aldo-keto reductase product benzo[a]pyrene-7,8-dione. J Biol Chem 2009;284:29725–29734. [PubMed: 19726680]

99. Amstad P, Crawford D, Muehlematter D, Zbinden I, Larsson R, Cerutti P. Oxidants stress induces the proto-oncogenes, C-fos and C-myc in mouse epidermal cells. Bull Cancer 1990;77:501–502. [PubMed: 2400824]

100. Amstad PA, Krupitza G, Cerutti PA. Mechanism of c-fos induction by active oxygen. Cancer Res 1992;52:3952–3960. [PubMed: 1617671]

101. Alexander A, Cai SL, Kim J, Nanez A, Sahin M, MacLean KH, Inoki K, Guan KL, Shen J, Person MD, Kusewitt D, Mills GB, Kastan MB, Walker CL. ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. Proc Natl Acad Sci U S A 107:4153–4158. [PubMed: 20160076]

102. Li L, Wang J, Ye RD, Shi G, Jin H, Tang X, Yi J. PML/RARalpha fusion protein mediates the unique sensitivity to arsenic cytotoxicity in acute promyelocytic leukemia cells: Mechanisms involve the impairment of cAMP signaling and the aberrant regulation of NADPH oxidase. J Cell Physiol 2008;217:486–493. [PubMed: 18636556]

103. Barlow CA, Kitiphongspattana K, Siddiqui N, Roe MW, Mossman BT, Lounsbury KM. Protein kinase A-mediated CREB phosphorylation is an oxidant-induced survival pathway in alveolar type II cells. Apoptosis 2008;13:681–692. [PubMed: 18392938]

104. Sun KH, de Pablo Y, Vincent F, Shah K. Deregulated Cdk5 promotes oxidative stress and mitochondrial dysfunction. J Neurochem 2008;107:265–278. [PubMed: 18691386]

105. Burch PM, Heintz NH. Redox regulation of cell-cycle re-entry: cyclin D1 as a primary target for the mitogenic effects of reactive oxygen and nitrogen species. Antioxid Redox Signal 2005;7:741–751. [PubMed: 15890020]

106. Franco R, Schoneveld O, Georgakilas AG, Panayiotidis MI. Oxidative stress, DNA methylation and carcinogenesis. Cancer Lett 2008;266:6–11. [PubMed: 18372104]

107. Maynard S, Schurman SH, Harboe C, de Souza-Pinto NC, Bohr VA. Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis 2009;30:2–10. [PubMed: 18978338]

108. Goldkorn T, Ravid T, Khan EM. Life and death decisions: ceramide generation and EGF receptor trafficking are modulated by oxidative stress. Antioxid Redox Signal 2005;7:119–128. [PubMed: 15650401]

109. Wartenberg M, Schallenberg M, Hescheler J, Sauer H. Reactive oxygen species-mediated regulation of eNOS and iNOS expression in multicellular prostate tumor spheroids. Int J Cancer 2003;104:274–282. [PubMed: 12569550]

110. McCubrey JA, Lahair MM, Franklin RA. Reactive oxygen species-induced activation of the MAP kinase signaling pathways. Antioxid Redox Signal 2006;8:1775–1789. [PubMed: 16987031]

111. Kwon D, Choi C, Lee J, Kim KO, Kim JD, Kim SJ, Choi IH. Hydrogen peroxide triggers the expression of Fas/FasL in astrocytoma cell lines and augments apoptosis. J Neuroimmunol 2001;113:1–9. [PubMed: 11137571]

112. Goto T, Takano M. Transcriptional role of FOXO1 in drug resistance through antioxidant defense systems. Adv Exp Med Biol 2009;665:171–179. [PubMed: 20429424]

113. Kietzmann T, Gorlach A. Reactive oxygen species in the control of hypoxia-inducible factor-mediated gene expression. Semin Cell Dev Biol 2005;16:474–486. [PubMed: 15905109]

114. Ryter SW, Choi AM. Heme oxygenase-1: redox regulation of a stress protein in lung and cell culture models. Antioxid Redox Signal 2005;7:80–91. [PubMed: 15650398]

115. Kuga S, Otsuka T, Niiro H, Nunoi H, Nemoto Y, Nakano T, Ogo T, Umei T, Niho Y. Suppression of superoxide anion production by interleukin-10 is accompanied by a downregulation of the genes for subunit proteins of NADPH oxidase. Exp Hematol 1996;24:151–157. [PubMed: 8641336]

116. Svineng G, Ravuri C, Rikardsen O, Huseby NE, Winberg JO. The role of reactive oxygen species in integrin and matrix metalloproteinase expression and function. Connect Tissue Res 2008;49:197–202. [PubMed: 18661342]

117. Hellstrand K, Hermodsson S, Naredi P, Mellqvist UH, Brune M. Histamine and cytokine therapy. Acta Oncol 1998;37:347–353. [PubMed: 9743456]

118. Simon AR, Rai U, Fanburg BL, Cochran BH. Activation of the JAK-STAT pathway by reactive oxygen species. Am J Physiol 1998;275:C1640–1652. [PubMed: 9843726]

119. Lo YY, Wong JM, Cruz TF. Reactive oxygen species mediate cytokine activation of c-Jun NH2-terminal kinases. J Biol Chem 1996;271:15703–15707. [PubMed: 8663189]

120. Skinner AM, Turker MS. Oxidative mutagenesis, mismatch repair, and aging. Sci Aging Knowledge Environ 2005;2005

121. Byun YJ, Kim SK, Kim YM, Chae GT, Jeong SW, Lee SB. Hydrogen peroxide induces autophagic cell death in C6 glioma cells via BNIP3-mediated suppression of the mTOR pathway. Neurosci Lett 2009;461:131–135. [PubMed: 19539716]

122. Venugopal R, Jaiswal AK. Nrf1 and Nrf2 positively and c-Fos and Fra1 negatively regulate the human antioxidant response element-mediated expression of NAD(P)H:quinone oxidoreductase1 gene. Proc Natl Acad Sci U S A 1996;93:14960–14965. [PubMed: 8962164]

123. Gloire G, Legrand-Poels S, Piette J. NF-kappaB activation by reactive oxygen species: fifteen years later. Biochem Pharmacol 2006;72:1493–1505. [PubMed: 16723122]

124. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, Oyake T, Hayashi N, Satoh K, Hatayama I, Yamamoto M, Nabeshima Y. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun 1997;236:313–322. [PubMed: 9240432]

125. Clerkin JS, Naughton R, Quiney C, Cotter TG. Mechanisms of ROS modulated cell survival during carcinogenesis. Cancer Lett 2008;266:30–36. [PubMed: 18372105]

126. Torres M, Forman HJ. Redox signaling and the MAP kinase pathways. Biofactors 2003;17:287–296. [PubMed: 12897450]

127. Han ES, Muller FL, Perez VI, Qi W, Liang H, Xi L, Fu C, Doyle E, Hickey M, Cornell J, Epstein CJ, Roberts LJ, Van Remmen H, Richardson A. The in vivo gene expression signature of oxidative stress. Physiol Genomics 2008;34:112–126. [PubMed: 18445702]

128. Tishler RB, Calderwood SK, Coleman CN, Price BD. Increases in sequence specific DNA binding by p53 following treatment with chemotherapeutic and DNA damaging agents. Cancer Res 1993;53:2212–2216. [PubMed: 8485705]

129. Kanthasamy AG, Kitazawa M, Kanthasamy A, Anantharam V. Role of proteolytic activation of protein kinase Cdelta in oxidative stress-induced apoptosis. Antioxid Redox Signal 2003;5:609–620. [PubMed: 14580317]

130. Rusyn I, Rose ML, Bojes HK, Thurman RG. Novel role of oxidants in the molecular mechanism of action of peroxisome proliferators. Antioxid Redox Signal 2000;2:607–621. [PubMed: 11229371]

131. Cao J, Schulte J, Knight A, Leslie NR, Zagozdzon A, Bronson R, Manevich Y, Beeson C, Neumann CA. Prdx1 inhibits tumorigenesis via regulating PTEN/AKT activity. EMBO J 2009;28:1505–1517. [PubMed: 19369943]

132. Chiarugi P. PTPs versus PTKs: the redox side of the coin. Free Radic Res 2005;39:353–364. [PubMed: 16028361]

133. Ammendola R, Mesuraca M, Russo T, Cimino F. The DNA-binding efficiency of Sp1 is affected by redox changes. Eur J Biochem 1994;225:483–489. [PubMed: 7925470]

134. Ushio-Fukai M, Alexander RW. Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase. Mol Cell Biochem 2004;264:85–97. [PubMed: 15544038]

135. Funato Y, Michiue T, Asashima M, Miki H. The thioredoxin-related redox-regulating protein nucleoredoxin inhibits Wnt-beta-catenin signalling through dishevelled. Nat Cell Biol 2006;8:501–508. [PubMed: 16604061]

136. Korswagen HC. Regulation of the Wnt/beta-catenin pathway by redox signaling. Dev Cell 2006;10:687–688. [PubMed: 16740470]

137. Droge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47–95. [PubMed: 11773609]

138. Kerr LD, Inoue J, Verma IM. Signal transduction: the nuclear target. Curr Opin Cell Biol 1992;4:496–501. [PubMed: 1497922]

139. Shaulian E, Karin M. AP-1 as a regulator of cell life and death. Nat Cell Biol 2002;4:E131–136. [PubMed: 11988758]

140. Gorlach A, Kietzmann T. Superoxide and derived reactive oxygen species in the regulation of hypoxia-inducible factors. Methods Enzymol 2007;435:421–446. [PubMed: 17998067]

141. Liu B, Chen Y, St Clair DK. ROS and p53: a versatile partnership. Free Radic Biol Med 2008;44:1529–1535. [PubMed: 18275858]

142. Mantovani A. Cancer: inflammation by remote control. Nature 2005;435:752–753. [PubMed: 15944689]

143. Ames BN, Gold LS. Animal cancer tests and cancer prevention. J Natl Cancer Inst Monogr 1992:125–132. [PubMed: 1616796]

144. Guyton KZ, Kensler TW. Oxidative mechanisms in carcinogenesis. Br Med Bull 1993;49:523–544. [PubMed: 8221020]

145. Schulte-Hermann R, Timmermann-Trosiener I, Barthel G, Bursch W. DNA synthesis, apoptosis, and phenotypic expression as determinants of growth of altered foci in rat liver during phenobarbital promotion. Cancer Res 1990;50:5127–5135. [PubMed: 2379175]

146. Klaunig JE, Xu Y, Isenberg JS, Bachowski S, Kolaja KL, Jiang J, Stevenson DE, Walborg EF Jr. The role of oxidative stress in chemical carcinogenesis. Environ Health Perspect 1998;106 1:289–295. [PubMed: 9539021]

147. Oshima H, B H. Chronic infectious and inflammation process as cancer risk factors: possible role of nitric oxide in carcinogenesis. Mutat Res 1994;305:253–264. [PubMed: 7510036]

148. Rosin MP, Saad el Din Zaki S, Ward AJ, Anwar WA. Involvement of inflammatory reactions and elevated cell proliferation in the development of bladder cancer in schistosomiasis patients. Mutat Res 1994;305:283–292. [PubMed: 7510039]

149. Weitzman SA, Gordon LI. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. Blood 1990;76:655–663. [PubMed: 2200535]

150. Frenkel K. Carcinogen-mediated oxidant formation and oxidative DNA damage. Pharmacol Ther 1992;53:127–166. [PubMed: 1641400]

151. Shacter E, Beecham EJ, Covey JM, Kohn KW, Potter M. Activated neutrophils induce prolonged DNA damage in neighboring cells. Carcinogenesis 1988;9:2297–2304. [PubMed: 2847879]

152. Wei H, Frenkel K. Suppression of tumor promoter-induced oxidative events and DNA damage in vivo by sarcophytol A: a possible mechanism of antipromotion. Cancer Res 1992;52:2298–2303. [PubMed: 1559232]

153. Hattori-Nakakuki Y, Nishigori C, Okamoto K, Imamura S, Hiai H, Toyokuni S. Formation of 8-hydroxy-2′-deoxyguanosine in epidermis of hairless mice exposed to near-UV. Biochem Biophys Res Commun 1994;201:1132–1139. [PubMed: 8024554]

154. Weitberg AB. Effect of combinations of antioxidants on phagocyte-induced sister-chromatid exchanges. Mutat Res 1989;224:1–4. [PubMed: 2549410]

155. Yamashina K, Miller BE, Heppner GH. Macrophage-mediated induction of drug-resistant variants in a mouse mammary tumor cell line. Cancer Res 1986;46:2396–2401. [PubMed: 3084067]

156. Dizdaroglu M, Olinski R, Doroshow JH, Akman SA. Modification of DNA bases in chromatin of intact target human cells by activated human polymorphonuclear leukocytes. Cancer Res 1993;53:1269–1272. [PubMed: 8383005]

157. Trush MA, Twerdok LE, Esterline RL. Comparison of oxidant activities and the activation of benzo(a)pyrene-7,8-dihydrodiol by polymorphonuclear leucocytes from human, rat and mouse. Xenobiotica 1990;20:925–932. [PubMed: 2173286]

158. Trush MA, Kensler TW. An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Radic Biol Med 1991;10:201–209. [PubMed: 1864525]

159. Jackson AL, Loeb LA. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. Mutat Res 2001;477:7–21. [PubMed: 11376682]

160. Storz P. Reactive oxygen species in tumor progression. Front Biosci 2005;10:1881–1896. [PubMed: 15769673]

161. Kroncke KD. Nitrosative stress and transcription. Biol Chem 2003;384:1365–1377. [PubMed: 14669980]

162. Cerutti PA, Trump BF. Inflammation and oxidative stress in carcinogenesis. Cancer Cells 1991;3:1–7. [PubMed: 2025490]

163. Cerutti PA. Prooxidant states and tumor promotion. Science 1985;227:375–381. [PubMed: 2981433]

164. Slaga TJ, Klein-Szanto AJ, Triplett LL, Yotti LP, Trosko KE. Skin tumor-promoting activity of benzoyl peroxide, a widely used free radical-generating compound. Science 1981;213:1023–1025. [PubMed: 6791284]

165. Kamata T. Roles of Nox1 and other Nox isoforms in cancer development. Cancer Sci 2009;100:1382–1388. [PubMed: 19493276]

166. Los M, Maddika S, Erb B, Schulze-Osthoff K. Switching Akt: from survival signaling to deadly response. Bioessays 2009;31:492–495. [PubMed: 19319914]

167. Azad MB, Chen Y, Gibson SB. Regulation of autophagy by reactive oxygen species (ROS): implications for cancer progression and treatment. Antioxid Redox Signal 2009;11:777–790. [PubMed: 18828708]

168. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell 2007;129:1261–1274. [PubMed: 17604717]

169. Plas DR, Thompson CB. Akt-dependent transformation: there is more to growth than just surviving. Oncogene 2005;24:7435–7442. [PubMed: 16288290]

170. Muller JM, Cahill MA, Rupec RA, Baeuerle PA, Nordheim A. Antioxidants as well as oxidants activate c-fos via Ras-dependent activation of extracellular-signal-regulated kinase 2 and Elk-1. Eur J Biochem 1997;244:45–52. [PubMed: 9063444]

171. Pennington JD, Wang TJ, Nguyen P, Sun L, Bisht K, Smart D, Gius D. Redox-sensitive signaling factors as a novel molecular targets for cancer therapy. Drug Resist Updat 2005;8:322–330. [PubMed: 16230045]

172. Xia Z, Dickens M, Raingeaud J, Davis RJ, Greenberg ME. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. Science 1995;270:1326–1331. [PubMed: 7481820]

173. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature 2001;410:37–40. [PubMed: 11242034]

174. Gupta S, Campbell D, Derijard B, Davis RJ. Transcription factor ATF2 regulation by the JNK signal transduction pathway. Science 1995;267:389–393. [PubMed: 7824938]

175. Karin M. The regulation of AP-1 activity by mitogen-activated protein kinases. J Biol Chem 1995;270:16483–16486. [PubMed: 7622446]

176. Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol 1996;14:649–683. [PubMed: 8717528]

177. Baeuerle PA, Lenardo M, Pierce JW, Baltimore D. Phorbol-ester-induced activation of the NF-kappa B transcription factor involves dissociation of an apparently cytoplasmic NF-kappa B/inhibitor complex. Cold Spring Harb Symp Quant Biol 1988;53(Pt 2):789–798. [PubMed: 3076097]

178. Li N, Karin M. Ionizing radiation and short wavelength UV activate NF-kappaB through two distinct mechanisms. Proc Natl Acad Sci U S A 1998;95:13012–13017. [PubMed: 9789032]

179. Rath PC, Aggarwal BB. Antiproliferative effects of IFN-alpha correlate with the downregulation of nuclear factor-kappa B in human Burkitt lymphoma Daudi cells. J Interferon Cytokine Res 2001;21:523–528. [PubMed: 11506747]

180. Bours V, Dejardin E, Goujon-Letawe F, Merville MP, Castronovo V. The NF-kappa B transcription factor and cancer: high expression of NF-kappa B- and I kappa B-related proteins in tumor cell lines. Biochem Pharmacol 1994;47:145–149. [PubMed: 8311838]

181. Delhalle S, Deregowski V, Benoit V, Merville MP, Bours V. NF-kappaB-dependent MnSOD expression protects adenocarcinoma cells from TNF-alpha-induced apoptosis. Oncogene 2002;21:3917–3924. [PubMed: 12032830]

182. Pham CG, Bubici C, Zazzeroni F, Papa S, Jones J, Alvarez K, Jayawardena S, De Smaele E, Cong R, Beaumont C, Torti FM, Torti SV, Franzoso G. Ferritin heavy chain upregulation by NF-

kappaB inhibits TNFalpha-induced apoptosis by suppressing reactive oxygen species. Cell 2004;119:529–542. [PubMed: 15537542]

183. Schulze-Osthoff K, Ferrari D, Los M, Wesselborg S, Peter ME. Apoptosis signaling by death receptors. Eur J Biochem 1998;254:439–459. [PubMed: 9688254]
184. Giri DK, Aggarwal BB. Constitutive activation of NF-kappaB causes resistance to apoptosis in human cutaneous T cell lymphoma HuT-78 cells. Autocrine role of tumor necrosis factor and reactive oxygen intermediates. J Biol Chem 1998;273:14008–14014. [PubMed: 9593751]
185. Nomura M, Ma W, Chen N, Bode AM, Dong Z. Inhibition of 12-O-tetradecanoylphorbol-13-acetate-induced NF-kappaB activation by tea polyphenols, (-)-epigallocatechin gallate and theaflavins. Carcinogenesis 2000;21:1885–1890. [PubMed: 11023547]
186. Schulze-Osthoff K, Bauer MK, Vogt M, Wesselborg S. Oxidative stress and signal transduction. Int J Vitam Nutr Res 1997;67:336–342. [PubMed: 9350475]
187. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med 1990;8:583–599. [PubMed: 2193855]
188. Schmidt KN, Amstad P, Cerutti P, Baeuerle PA. The roles of hydrogen peroxide and superoxide as messengers in the activation of transcription factor NF-kappa B. Chem Biol 1995;2:13–22. [PubMed: 9383399]
189. Kass GE, Duddy SK, Orrenius S. Activation of hepatocyte protein kinase C by redox-cycling quinones. Biochem J 1989;260:499–507. [PubMed: 2764885]
190. Larsson R, Cerutti P. Translocation and enhancement of phosphotransferase activity of protein kinase C following exposure in mouse epidermal cells to oxidants. Cancer Res 1989;49:5627–5632. [PubMed: 2507133]
191. Konishi H, Matsuzaki H, Tanaka M, Takemura Y, Kuroda S, Ono Y, Kikkawa U. Activation of protein kinase B (Akt/RAC-protein kinase) by cellular stress and its association with heat shock protein Hsp27. FEBS Lett 1997;410:493–498. [PubMed: 9237690]
192. D'Souza RJ, Phillips HM, Jones PW, Strange RC, Aber GM. Interactions of hydrogen peroxide with interleukin-6 and platelet-derived growth factor in determining mesangial cell growth: effect of repeated oxidant stress. Clin Sci (Lond) 1993;85:747–751. [PubMed: 8287668]
193. Alliangana DM. Effects of beta-carotene, flavonoid quercitin and quinacrine on cell proliferation and lipid peroxidation breakdown products in BHK-21 cells. East Afr Med J 1996;73:752–757. [PubMed: 8997868]
194. Chang MC, Chan CP, Wang YJ, Lee PH, Chen LI, Tsai YL, Lin BR, Wang YL, Jeng JH. Induction of necrosis and apoptosis to KB cancer cells by sanguinarine is associated with reactive oxygen species production and mitochondrial membrane depolarization. Toxicol Appl Pharmacol 2007;218:143–151. [PubMed: 17196629]
195. Dypbukt JM, Ankarcrona M, Burkitt M, Sjoholm A, Strom K, Orrenius S, Nicotera P. Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin-secreting RINm5F cells. The role of intracellular polyamines. J Biol Chem 1994;269:30553–30560. [PubMed: 7982974]
196. Halliwell B. Oxidative stress and cancer: have we moved forward? Biochem J 2007;401:1–11. [PubMed: 17150040]
197. Kundu N, Zhang S, Fulton AM. Sublethal oxidative stress inhibits tumor cell adhesion and enhances experimental metastasis of murine mammary carcinoma. Clin Exp Metastasis 1995;13:16–22. [PubMed: 7820952]
198. Roebuck KA. Oxidant stress regulation of IL-8 and ICAM-1 gene expression: differential activation and binding of the transcription factors AP-1 and NF-kappaB (Review). Int J Mol Med 1999;4:223–230. [PubMed: 10425270]
199. Westermarck J, Kahari VM. Regulation of matrix metalloproteinase expression in tumor invasion. FASEB J 1999;13:781–792. [PubMed: 10224222]
200. Mori K, Shibanuma M, Nose K. Invasive potential induced under long-term oxidative stress in mammary epithelial cells. Cancer Res 2004;64:7464–7472. [PubMed: 15492271]
201. Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000;18:1135–1149. [PubMed: 10694567]

202. Rajagopalan S, Meng XP, Ramasamy S, Harrison DG, Galis ZS. Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro. Implications for atherosclerotic plaque stability. J Clin Invest 1996;98:2572–2579. [PubMed: 8958220]

203. Nelson KK, Melendez JA. Mitochondrial redox control of matrix metalloproteinases. Free Radic Biol Med 2004;37:768–784. [PubMed: 15304253]

204. Yoon SO, Park SJ, Yoon SY, Yun CH, Chung AS. Sustained production of H(2)O(2) activates pro-matrix metalloproteinase-2 through receptor tyrosine kinases/phosphatidylinositol 3-kinase/NF-kappa B pathway. J Biol Chem 2002;277:30271–30282. [PubMed: 12058032]

205. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70. [PubMed: 10647931]

206. Kollmar O, Rupertus K, Scheuer C, Junker B, Tilton B, Schilling MK, Menger MD. Stromal cell-derived factor-1 promotes cell migration and tumor growth of colorectal metastasis. Neoplasia 2007;9:862–870. [PubMed: 17971906]

207. Owen JD, Strieter R, Burdick M, Haghnegahdar H, Nanney L, Shattuck-Brandt R, Richmond A. Enhanced tumor-forming capacity for immortalized melanocytes expressing melanoma growth stimulatory activity/growth-regulated cytokine beta and gamma proteins. Int J Cancer 1997;73:94–103. [PubMed: 9334815]

208. Yuecheng Y, Xiaoyan X. Stromal-cell derived factor-1 regulates epithelial ovarian cancer cell invasion by activating matrix metalloproteinase-9 and matrix metalloproteinase-2. Eur J Cancer Prev 2007;16:430–435. [PubMed: 17923814]

209. Lu H, Ouyang W, Huang C. Inflammation, a key event in cancer development. Mol Cancer Res 2006;4:221–233. [PubMed: 16603636]

210. Wen DS, Zhu XL, Guan SM, Wu YM, Yu LL, Wu JZ. Silencing of CXCR4 inhibits the proliferation, adhesion, chemotaxis and invasion of salivary gland mucoepidermoid carcinoma Mc3 cells in vitro. Oral Oncol 2008;44:545–554. [PubMed: 17936060]

211. van Wetering S, van Buul JD, Quik S, Mul FP, Anthony EC, ten Klooster JP, Collard JG, Hordijk PL. Reactive oxygen species mediate Rac-induced loss of cell-cell adhesion in primary human endothelial cells. J Cell Sci 2002;115:1837–1846. [PubMed: 11956315]

212. Diers AR, Dranka BP, Ricart KC, Oh JY, Johnson MS, Zhou F, Pallero MA, Bodenstine TM, Murphy-Ullrich JE, Welch DR, Landar A. Modulation of mammary cancer cell migration by 15-deoxy-delta(12,14)-prostaglandin J(2): implications for anti-metastatic therapy. Biochem J 430:69–78. [PubMed: 20536428]

213. Stamatakis K, Perez-Sala D. Prostanoids with cyclopentenone structure as tools for the characterization of electrophilic lipid-protein interactomes. Ann N Y Acad Sci 2006;1091:548–570. [PubMed: 17341644]

214. Stamatakis K, Sanchez-Gomez FJ, Perez-Sala D. Identification of novel protein targets for modification by 15-deoxy-Delta12,14-prostaglandin J2 in mesangial cells reveals multiple interactions with the cytoskeleton. J Am Soc Nephrol 2006;17:89–98. [PubMed: 16291835]

215. Aldini G, Carini M, Vistoli G, Shibata T, Kusano Y, Gamberoni L, Dalle-Donne I, Milzani A, Uchida K. Identification of actin as a 15-deoxy-Delta12,14-prostaglandin J2 target in neuroblastoma cells: mass spectrometric, computational, and functional approaches to investigate the effect on cytoskeletal derangement. Biochemistry 2007;46:2707–2718. [PubMed: 17297918]

216. Cheng GC, Schulze PC, Lee RT, Sylvan J, Zetter BR, Huang H. Oxidative stress and thioredoxin-interacting protein promote intravasation of melanoma cells. Exp Cell Res 2004;300:297–307. [PubMed: 15474995]

217. Folkman J. Tumor angiogenesis. Adv Cancer Res 1985;43:175–203. [PubMed: 2581424]

218. Aruoma OI, Halliwell B. Superoxide-dependent and ascorbate-dependent formation of hydroxyl radicals from hydrogen peroxide in the presence of iron. Are lactoferrin and transferrin promoters of hydroxyl-radical generation? Biochem J 1987;241:273–278. [PubMed: 3032157]

219. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991;64:327–336. [PubMed: 1703045]

220. North S, Moenner M, Bikfalvi A. Recent developments in the regulation of the angiogenic switch by cellular stress factors in tumors. Cancer Lett 2005;218:1–14. [PubMed: 15639335]

221. Bergers G, Benjamin LE. Tumorigenesis and the angiogenic switch. Nat Rev Cancer 2003;3:401–410. [PubMed: 12778130]

222. Chandel NS, Maltepe E, Goldwasser E, Mathieu CE, Simon MC, Schumacker PT. Mitochondrial reactive oxygen species trigger hypoxia-induced transcription. Proc Natl Acad Sci U S A 1998;95:11715–11720. [PubMed: 9751731]

223. Mazure NM, Chen EY, Yeh P, Laderoute KR, Giaccia AJ. Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. Cancer Res 1996;56:3436–3440. [PubMed: 8758908]

224. Okada F, Rak JW, Croix BS, Lieubeau B, Kaya M, Roncari L, Shirasawa S, Sasazuki T, Kerbel RS. Impact of oncogenes in tumor angiogenesis: mutant K-ras up-regulation of vascular endothelial growth factor/vascular permeability factor is necessary, but not sufficient for tumorigenicity of human colorectal carcinoma cells. Proc Natl Acad Sci U S A 1998;95:3609–3614. [PubMed: 9520413]

225. Van Meir EG, Polverini PJ, Chazin VR, Su Huang HJ, de Tribolet N, Cavenee WK. Release of an inhibitor of angiogenesis upon induction of wild type p53 expression in glioblastoma cells. Nat Genet 1994;8:171–176. [PubMed: 7531056]

226. Longo R, Sarmiento R, Fanelli M, Capaccetti B, Gattuso D, Gasparini G. Anti-angiogenic therapy: rationale, challenges and clinical studies. Angiogenesis 2002;5:237–256. [PubMed: 12906317]

227. Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med 1995;1:1024–1028. [PubMed: 7489357]

228. Eatock MM, Schatzlein A, Kaye SB. Tumour vasculature as a target for anticancer therapy. Cancer Treat Rev 2000;26:191–204. [PubMed: 10814561]

229. Monte M, Davel LE, Sacerdote de Lustig E. Hydrogen peroxide is involved in lymphocyte activation mechanisms to induce angiogenesis. Eur J Cancer 1997;33:676–682. [PubMed: 9274453]

230. Monte M, Davel LE, de Lustig ES. Inhibition of lymphocyte-induced angiogenesis by free radical scavengers. Free Radic Biol Med 1994;17:259–266. [PubMed: 7526998]

231. Harris AL. Hypoxia--a key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38–47. [PubMed: 11902584]

232. Hockel M, Schlenger K, Hockel S, Vaupel P. Hypoxic cervical cancers with low apoptotic index are highly aggressive. Cancer Res 1999;59:4525–4528. [PubMed: 10493500]

233. Hockel M, Vaupel P. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001;93:266–276. [PubMed: 11181773]

234. Roessner A, Kuester D, Malfertheiner P, Schneider-Stock R. Oxidative stress in ulcerative colitis-associated carcinogenesis. Pathol Res Pract 2008;204:511–524. [PubMed: 18571874]

235. Martey CA, Pollock SJ, Turner CK, O'Reilly KM, Baglole CJ, Phipps RP, Sime PJ. Cigarette smoke induces cyclooxygenase-2 and microsomal prostaglandin E2 synthase in human lung fibroblasts: implications for lung inflammation and cancer. Am J Physiol Lung Cell Mol Physiol 2004;287:L981–991. [PubMed: 15234907]

236. Garcea G, Dennison AR, Steward WP, Berry DP. Role of inflammation in pancreatic carcinogenesis and the implications for future therapy. Pancreatology 2005;5:514–529. [PubMed: 16110250]

237. Murphy SJ, Anderson LA, Johnston BT, Fitzpatrick DA, Watson PR, Monaghan P, Murray LJ. Have patients with esophagitis got an increased risk of adenocarcinoma? Results from a population-based study. World J Gastroenterol 2005;11:7290–7295. [PubMed: 16437630]

238. de Visser KE, Jonkers J. Towards understanding the role of cancer-associated inflammation in chemoresistance. Curr Pharm Des 2009;15:1844–1853. [PubMed: 19519427]

239. Borst P, Jonkers J, Rottenberg S. What makes tumors multidrug resistant? Cell Cycle 2007;6:2782–2787. [PubMed: 17998803]

240. Schmitt CA, Fridman JS, Yang M, Lee S, Baranov E, Hoffman RM, Lowe SW. A senescence program controlled by p53 and p16INK4a contributes to the outcome of cancer therapy. Cell 2002;109:335–346. [PubMed: 12015983]

241. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Targeting multidrug resistance in cancer. Nat Rev Drug Discov 2006;5:219–234. [PubMed: 16518375]

242. Voorzanger-Rousselot N, Alberti L, Blay JY. CD40L induces multidrug resistance to apoptosis in breast carcinoma and lymphoma cells through caspase independent and dependent pathways. BMC Cancer 2006;6:75. [PubMed: 16545138]

243. Ho EA, Piquette-Miller M. Regulation of multidrug resistance by proinflammatory cytokines. Curr Cancer Drug Targets 2006;6:295–311. [PubMed: 16848721]

244. Petrovic V, Teng S, Piquette-Miller M. Regulation of drug transporters during infection and inflammation. Mol Interv 2007;7:99–111. [PubMed: 17468390]

245. Hartmann G, Vassileva V, Piquette-Miller M. Impact of endotoxin-induced changes in P-glycoprotein expression on disposition of doxorubicin in mice. Drug Metab Dispos 2005;33:820–828. [PubMed: 15778272]

246. Blokzijl H, van Steenpaal A, Vander Borght S, Bok LI, Libbrecht L, Tamminga M, Geuken M, Roskams TA, Dijkstra G, Moshage H, Jansen PL, Faber KN. Up-regulation and cytoprotective role of epithelial multidrug resistance-associated protein 1 in inflammatory bowel disease. J Biol Chem 2008;283:35630–35637. [PubMed: 18838379]

247. Bottero V, Busuttil V, Loubat A, Magne N, Fischel JL, Milano G, Peyron JF. Activation of nuclear factor kappaB through the IKK complex by the topoisomerase poisons SN38 and doxorubicin: a brake to apoptosis in HeLa human carcinoma cells. Cancer Res 2001;61:7785–7791. [PubMed: 11691793]

248. Das KC, White CW. Activation of NF-kappaB by antineoplastic agents. Role of protein kinase C. J Biol Chem 1997;272:14914–14920. [PubMed: 9169462]

249. Piret B, Piette J. Topoisomerase poisons activate the transcription factor NF-kappaB in ACH-2 and CEM cells. Nucleic Acids Res 1996;24:4242–4248. [PubMed: 8932379]

250. Ahn KS, Sethi G, Aggarwal BB. Nuclear factor-kappa B: from clone to clinic. Curr Mol Med 2007;7:619–637. [PubMed: 18045141]

251. Raju U, Gumin GJ, Noel F, Tofilon PJ. IkappaBalpha degradation is not a requirement for the X-ray-induced activation of nuclear factor kappaB in normal rat astrocytes and human brain tumour cells. Int J Radiat Biol 1998;74:617–624. [PubMed: 9848280]

252. Chen R, Alvero AB, Silasi DA, Kelly MG, Fest S, Visintin I, Leiser A, Schwartz PE, Rutherford T, Mor G. Regulation of IKKbeta by miR-199a affects NF-kappaB activity in ovarian cancer cells. Oncogene 2008;27:4712–4723. [PubMed: 18408758]

253. Kelly MG, Alvero AB, Chen R, Silasi DA, Abrahams VM, Chan S, Visintin I, Rutherford T, Mor G. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. Cancer Res 2006;66:3859–3868. [PubMed: 16585214]

254. Braunstein S, Formenti SC, Schneider RJ. Acquisition of stable inducible upregulation of nuclear factor-kappaB by tumor necrosis factor exposure confers increased radiation resistance without increased transformation in breast cancer cells. Mol Cancer Res 2008;6:78–88. [PubMed: 18234964]

255. Douglas KT. Mechanism of action of glutathione-dependent enzymes. Adv Enzymol Relat Areas Mol Biol 1987;59:103–167. [PubMed: 2880477]

256. Sau A, Pellizzari Tregno F, Valentino F, Federici G, Caccuri AM. Glutathione transferases and development of new principles to overcome drug resistance. Arch Biochem Biophys 500:116–122. [PubMed: 20494652]

257. Hayes JD, Flanagan JU, Jowsey IR. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005;45:51–88. [PubMed: 15822171]

258. Lau A, Villeneuve NF, Sun Z, Wong PK, Zhang DD. Dual roles of Nrf2 in cancer. Pharmacol Res 2008;58:262–270. [PubMed: 18838122]

259. Wang XJ, Sun Z, Villeneuve NF, Zhang S, Zhao F, Li Y, Chen W, Yi X, Zheng W, Wondrak GT, Wong PK, Zhang DD. Nrf2 enhances resistance of cancer cells to chemotherapeutic drugs, the dark side of Nrf2. Carcinogenesis 2008;29:1235–1243. [PubMed: 18413364]

260. Cho JM, Manandhar S, Lee HR, Park HM, Kwak MK. Role of the Nrf2-antioxidant system in cytotoxicity mediated by anticancer cisplatin: implication to cancer cell resistance. Cancer Lett 2008;260:96–108. [PubMed: 18036733]

261. Padmanabhan B, Tong KI, Ohta T, Nakamura Y, Scharlock M, Ohtsuji M, Kang MI, Kobayashi A, Yokoyama S, Yamamoto M. Structural basis for defects of Keap1 activity provoked by its point mutations in lung cancer. Mol Cell 2006;21:689–700. [PubMed: 16507366]

262. Singh A, Misra V, Thimmulappa RK, Lee H, Ames S, Hoque MO, Herman JG, Baylin SB, Sidransky D, Gabrielson E, Brock MV, Biswal S. Dysfunctional KEAP1-NRF2 interaction in non-small-cell lung cancer. PLoS Med 2006;3:e420. [PubMed: 17020408]

263. Jozkowicz A, Was H, Dulak J. Heme oxygenase-1 in tumors: is it a false friend? Antioxid Redox Signal 2007;9:2099–2117. [PubMed: 17822372]

264. Brigelius-Flohe R. Selenium compounds and selenoproteins in cancer. Chem Biodivers 2008;5:389–395. [PubMed: 18357548]

265. Kim JH, Bogner PN, Ramnath N, Park Y, Yu J, Park YM. Elevated peroxiredoxin 1, but not NF-E2-related factor 2, is an independent prognostic factor for disease recurrence and reduced survival in stage I non-small cell lung cancer. Clin Cancer Res 2007;13:3875–3882. [PubMed: 17606720]

266. Kim YJ, Ahn JY, Liang P, Ip C, Zhang Y, Park YM. Human prx1 gene is a target of Nrf2 and is up-regulated by hypoxia/reoxygenation: implication to tumor biology. Cancer Res 2007;67:546–554. [PubMed: 17234762]

267. Persons DL, Yazlovitskaya EM, Cui W, Pelling JC. Cisplatin-induced activation of mitogen-activated protein kinases in ovarian carcinoma cells: inhibition of extracellular signal-regulated kinase activity increases sensitivity to cisplatin. Clin Cancer Res 1999;5:1007–1014. [PubMed: 10353733]

268. Steinmetz R, Wagoner HA, Zeng P, Hammond JR, Hannon TS, Meyers JL, Pescovitz OH. Mechanisms regulating the constitutive activation of the extracellular signal-regulated kinase (ERK) signaling pathway in ovarian cancer and the effect of ribonucleic acid interference for ERK1/2 on cancer cell proliferation. Mol Endocrinol 2004;18:2570–2582. [PubMed: 15243131]

269. Camps M, Nichols A, Arkinstall S. Dual specificity phosphatases: a gene family for control of MAP kinase function. FASEB J 2000;14:6–16. [PubMed: 10627275]

270. Keyse SM. Protein phosphatases and the regulation of mitogen-activated protein kinase signalling. Curr Opin Cell Biol 2000;12:186–192. [PubMed: 10712927]

271. Nichols A, Camps M, Gillieron C, Chabert C, Brunet A, Wilsbacher J, Cobb M, Pouyssegur J, Shaw JP, Arkinstall S. Substrate recognition domains within extracellular signal-regulated kinase mediate binding and catalytic activation of mitogen-activated protein kinase phosphatase-3. J Biol Chem 2000;275:24613–24621. [PubMed: 10811804]

272. Kim JJ, Tannock IF. Repopulation of cancer cells during therapy: an important cause of treatment failure. Nat Rev Cancer 2005;5:516–525. [PubMed: 15965493]

273. Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. Science 1984;223:594–597. [PubMed: 6695170]

274. Orlowski RZ, Baldwin AS Jr. NF-kappaB as a therapeutic target in cancer. Trends Mol Med 2002;8:385–389. [PubMed: 12127724]

275. Fan M, Ahmed KM, Coleman MC, Spitz DR, Li JJ. Nuclear factor-kappaB and manganese superoxide dismutase mediate adaptive radioresistance in low-dose irradiated mouse skin epithelial cells. Cancer Res 2007;67:3220–3228. [PubMed: 17409430]

276. Guo G, Yan-Sanders Y, Lyn-Cook BD, Wang T, Tamae D, Ogi J, Khaletskiy A, Li Z, Weydert C, Longmate JA, Huang TT, Spitz DR, Oberley LW, Li JJ. Manganese superoxide dismutase-mediated gene expression in radiation-induced adaptive responses. Mol Cell Biol 2003;23:2362–2378. [PubMed: 12640121]

277. Li Z, Xia L, Lee LM, Khaletskiy A, Wang J, Wong JY, Li JJ. Effector genes altered in MCF-7 human breast cancer cells after exposure to fractionated ionizing radiation. Radiat Res 2001;155:543–553. [PubMed: 11260656]

278. Izzo JG, Malhotra U, Wu TT, Ensor J, Luthra R, Lee JH, Swisher SG, Liao Z, Chao KS, Hittelman WN, Aggarwal BB, Ajani JA. Association of activated transcription factor nuclear factor kappab with chemoradiation resistance and poor outcome in esophageal carcinoma. J Clin Oncol 2006;24:748–754. [PubMed: 16401681]

279. Sandur SK, Deorukhkar A, Pandey MK, Pabon AM, Shentu S, Guha S, Aggarwal BB, Krishnan S. Curcumin modulates the radiosensitivity of colorectal cancer cells by suppressing constitutive and inducible NF-kappaB activity. Int J Radiat Oncol Biol Phys 2009;75:534–542. [PubMed: 19735878]

280. De Ridder M, Verellen D, Verovski V, Storme G. Hypoxic tumor cell radiosensitization through nitric oxide. Nitric Oxide 2008;19:164–169. [PubMed: 18474256]

281. Janssens MY, Verovski VN, Van den Berge DL, Monsaert C, Storme GA. Radiosensitization of hypoxic tumour cells by S-nitroso-N-acetylpenicillamine implicates a bioreductive mechanism of nitric oxide generation. Br J Cancer 1999;79:1085–1089. [PubMed: 10098740]

282. Kimura K, Bowen C, Spiegel S, Gelmann EP. Tumor necrosis factor-alpha sensitizes prostate cancer cells to gamma-irradiation-induced apoptosis. Cancer Res 1999;59:1606–1614. [PubMed: 10197636]

283. Mitchell JB, Wink DA, DeGraff W, Gamson J, Keefer LK, Krishna MC. Hypoxic mammalian cell radiosensitization by nitric oxide. Cancer Res 1993;53:5845–5848. [PubMed: 8261391]

284. Sersa G, Willingham V, Milas L. Anti-tumor effects of tumor necrosis factor alone or combined with radiotherapy. Int J Cancer 1988;42:129–134. [PubMed: 3391701]

285. Verovski VN, Van den Berge DL, Soete GA, Bols BL, Storme GA. Intrinsic radiosensitivity of human pancreatic tumour cells and the radiosensitising potency of the nitric oxide donor sodium nitroprusside. Br J Cancer 1996;74:1734–1742. [PubMed: 8956786]

286. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 1997;3:730–737. [PubMed: 9212098]

287. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003;100:3983–3988. [PubMed: 12629218]

288. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Res 2005;65:10946–10951. [PubMed: 16322242]

289. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. Cancer Res 2007;67:1030–1037. [PubMed: 17283135]

290. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. Nature 2007;445:111–115. [PubMed: 17122771]

291. Schatton T, Murphy GF, Frank NY, Yamaura K, Waaga-Gasser AM, Gasser M, Zhan Q, Jordan S, Duncan LM, Weishaupt C, Fuhlbrigge RC, Kupper TS, Sayegh MH, Frank MH. Identification of cells initiating human melanomas. Nature 2008;451:345–349. [PubMed: 18202660]

292. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature 2004;432:396–401. [PubMed: 15549107]

293. Szotek PP, Pieretti-Vanmarcke R, Masiakos PT, Dinulescu DM, Connolly D, Foster R, Dombkowski D, Preffer F, Maclaughlin DT, Donahoe PK. Ovarian cancer side population defines cells with stem cell-like characteristics and Mullerian Inhibiting Substance responsiveness. Proc Natl Acad Sci U S A 2006;103:11154–11159. [PubMed: 16849428]

294. Korkaya H, Wicha MS. Selective targeting of cancer stem cells: a new concept in cancer therapeutics. BioDrugs 2007;21:299–310. [PubMed: 17896836]

295. Diehn M, Cho RW, Lobo NA, Kalisky T, Dorie MJ, Kulp AN, Qian D, Lam JS, Ailles LE, Wong M, Joshua B, Kaplan MJ, Wapnir I, Dirbas FM, Somlo G, Garberoglio C, Paz B, Shen J, Lau SK, Quake SR, Brown JM, Weissman IL, Clarke MF. Association of reactive oxygen species levels and radioresistance in cancer stem cells. Nature 2009;458:780–783. [PubMed: 19194462]

296. Baba T, Convery PA, Matsumura N, Whitaker RS, Kondoh E, Perry T, Huang Z, Bentley RC, Mori S, Fujii S, Marks JR, Berchuck A, Murphy SK. Epigenetic regulation of CD133 and tumorigenicity of CD133+ ovarian cancer cells. Oncogene 2009;28:209–218. [PubMed: 18836486]

297. Ma S, Lee TK, Zheng BJ, Chan KW, Guan XY. CD133+ HCC cancer stem cells confer chemoresistance by preferential expression of the Akt/PKB survival pathway. Oncogene 2008;27:1749–1758. [PubMed: 17891174]

298. Rhee HW, Zhau HE, Pathak S, Multani AS, Pennanen S, Visakorpi T, Chung LW. Permanent phenotypic and genotypic changes of prostate cancer cells cultured in a three-dimensional rotating-wall vessel. In Vitro Cell Dev Biol Anim 2001;37:127–140. [PubMed: 11370803]

299. Thalmann GN, Anezinis PE, Chang SM, Zhau HE, Kim EE, Hopwood VL, Pathak S, von Eschenbach AC, Chung LW. Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. Cancer Res 1994;54:2577–2581. [PubMed: 8168083]

300. Josson S, Matsuoka Y, Chung LW, Zhau HE, Wang R. Tumor-stroma coevolution in prostate cancer progression and metastasis. Semin Cell Dev Biol 21:26–32. [PubMed: 19948237]

301. De Marzo AM, Nakai Y, Nelson WG. Inflammation, atrophy, and prostate carcinogenesis. Urol Oncol 2007;25:398–400. [PubMed: 17826659]

302. Sung SY, Hsieh CL, Law A, Zhau HE, Pathak S, Multani AS, Lim S, Coleman IM, Wu LC, Figg WD, Dahut WL, Nelson P, Lee JK, Amin MB, Lyles R, Johnstone PA, Marshall FF, Chung LW. Coevolution of prostate cancer and bone stroma in three-dimensional coculture: implications for cancer growth and metastasis. Cancer Res 2008;68:9996–10003. [PubMed: 19047182]

303. Chung LW, Baseman A, Assikis V, Zhau HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. J Urol 2005;173:10–20. [PubMed: 15592017]

304. Porta C, Larghi P, Rimoldi M, Totaro MG, Allavena P, Mantovani A, Sica A. Cellular and molecular pathways linking inflammation and cancer. Immunobiology 2009;214:761–777. [PubMed: 19616341]

305. Mantovani A, Sica A, Allavena P, Garlanda C, Locati M. Tumor-associated macrophages and the related myeloid-derived suppressor cells as a paradigm of the diversity of macrophage activation. Hum Immunol 2009;70:325–330. [PubMed: 19236898]

306. Sica A, Bronte V. Altered macrophage differentiation and immune dysfunction in tumor development. J Clin Invest 2007;117:1155–1166. [PubMed: 17476345]

307. Balkwill F, Charles KA, Mantovani A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005;7:211–217. [PubMed: 15766659]

308. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545. [PubMed: 11229684]

309. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436–444. [PubMed: 18650914]

310. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23:549–555. [PubMed: 12401408]

311. Hiratsuka S, Nakamura K, Iwai S, Murakami M, Itoh T, Kijima H, Shipley JM, Senior RM, Shibuya M. MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. Cancer Cell 2002;2:289–300. [PubMed: 12398893]

312. Surh YJ, Kundu JK, Na HK, Lee JS. Redox-sensitive transcription factors as prime targets for chemoprevention with anti-inflammatory and antioxidative phytochemicals. J Nutr 2005;135:2993S–3001S. [PubMed: 16317160]

313. Virgili F, Marino M. Regulation of cellular signals from nutritional molecules: a specific role for phytochemicals, beyond antioxidant activity. Free Radic Biol Med 2008;45:1205–1216. [PubMed: 18762244]

6. Abbreviations

Akt	AKT8 virus oncogene cellular homolog
AP-1	activator protein-1

APC	adenomatous polyposis coli
ATF-2	activating transcription factor-2
Bad	Bcl-XL/Bcl-2-associated death promoter
BH3	Bcl-2 homology3
BRCA1	breast cancer susceptibility gene 1
CDKN-2	cyclin-dependent kinase inhibitor-2
COX-2	cyclooxygenase-2
CCL5	CC chemokine ligand 5
CSCs	cancer stem cells
Cu-ZnSOD	copper-zinc superoxide dismutase
CXCL5	CXC chemokine lix 5
CXCR4	CXC chemokine receptor 4
ECM	extracellular matrix
EC-SOD	extracellular-superoxide dismutase
eNOS	endothelial nitric oxide synthase
ERK/MAPK	extracellular signal-regulated kinase/ mitogen-activated protein kinase
FGF	fibroblast growth factor
HIF-1α	hypoxia inducible factor-1α
Flk1/KDR	fetal liver kinase 1/ kinase insert domain receptor
GPx	glutathione peroxidase
GSH	glutathione
GSSG	glutathione disulphide
GTPase Rac1	guanosine triphosphatase Rac1
HER-2	human epidermal growth factor receptor-2
HGF/SF	hepatocyte growth factor/ scatter factor
HIF-1α	hypoxia-inducible factor-1α
hMLH1	human mutL homolog 1
HMOX-1	heme oxygenase-1
4-HNE	4-hydroxynonenal
H_2O_2	hydrogen peroxide
Hsp27	heat shock protein27
ICAM-1	intercellular adhesion molecule-1
IGF-1	Insulin like growth factor-1
IκBα	inhibitor of κBα
IL-1	interleukin-1
IL-6	interleukin-6

IL-8	interleukin-8
iNOS	inducible nitric oxide synthase
IFN	interferon
JNK	c-Jun N-terminal kinase
c-JUN	cellular Ju-nanna
KGF	keratinocyte growth factor
Keap1	Kelch-like ECH-associated protein 1
LPS	lipopolysaccharide
MDR	multidrug-resistance
MDM2	murine double minute 2
MKPs	mitogen-activated protein kinase phosphatases
MMPs	metalloproteinases
Mn-SOD	manganese-superoxide dismutase
MRP1	multidrug resistance-associated protein 1
Myc	avian myeloblastosis virus oncogene
MyD88	myeloid differentiation primary response gene 88
NAC	N-acetylcysteine
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NFAT	nuclear factor of activated T cells
NF-κB	nuclear factor κ B
NO	nitric oxide
Nox	NADPH oxidase
Nrf2	NF-E2 related factor-2
8-OHdG	8-hydroxydeoxyguanosine
p27Kip1	p27 kinase inhibitor protein
PDGF	platelet-derived growth factor
PGP	P-glycoprotein
PI3K	phosphoinositide 3- kinase
PKB/Akt	protein kinase B/AKT8 virus oncogene cellular homolog
PMA	phorbol 12-myristate 13- acetate
PPAR-γ	peroxisome proliferator-activated receptor-γ
PTEN	phosphatase and tensin homolog deleted from chromosome 10
Prx	peroxiredoxins
RAS	rat sarcoma viral oncogene
RAF	murine sarcoma 3611 oncogene
Rb	retinoblastoma protein

ROS	reactive oxygen species
RNS	reactive nitrogen species
SDF-1/CXCL12	stromal cell derived factor-1/ CXC chemokine ligand 12
SOD	superoxide dismutase
SRC	steroid receptor coactivator
STAT3	signal transducer and activator of transcription 3
TAM	tumor-associated macrophages
tBHQ	tertiary-butylhydroquinone
TGF-β	transforming growth factor-β
TLR	toll-like receptor
TNF	tumor necrosis factor
TSP-1	thrombospondin-1
TrxR	thioredoxin reductase
VEGF-A	vascular endothelial growth factor-A
Wnt	wint

Figure 1. Schematic representation of various activators and inhibitors of reactive oxygen species production

Figure 2. Schematic representation of various transcription factors that are modulated by reactive oxygen species

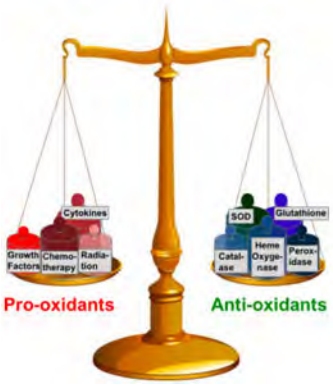

Figure 3. Model of a balance between pro-oxidants and anti-oxidants

Under normal conditions, anti-oxidants outbalance pro-oxidants, but under oxidative conditions, pro-oxidants prevail over anti-oxidants, which can lead to many inflammatory diseases including cancer.

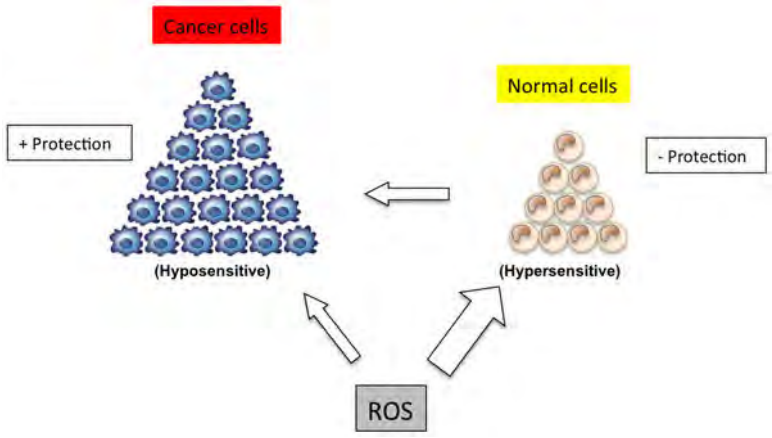

Figure 4. Model of the sensitivity of normal cells versus cancer cells to reactive oxygen species

Normal cells are hypersensitive to ROS if not adequately protected by anti-oxidant mechanisms, which may lead to cancer formation. Cancer cells, on the other hand, have upregulated antioxidant mechanisms (glutathione, SOD, catalase, and others) that will protect them against ROS, as can be observed in, for example, the case of radioresistance.

Table 1

A partial list of diseases that have been linked to reactive oxygen species

Disease	Reference
Acute Respiratory Distress Syndrome	[16]
Aging	[17]
Alzheimer	[18,19]
Atherosclerosis	[20]
Cancer	[21-23]
Cardiovascular Disease	[24,25]
Diabetes	[26]
Inflammation	[27]
Inflammatory Joint Disease	[28]
Neurological Disease	[29]
Obesity	[30,31]
Parkinson	[32,33]
Pulmonary fibrosis	[34,35]
Rheumatoid arthritis	[36]
Vascular Disease	[37,38]

Table 2
A partial list of cancers that have been linked to reactive oxygen species

Cancer	Reference
Bladder Cancer	[39]
Brain Tumor	[40]
Breast Cancer	[41]
Cervical Cancer	[42]
Gastric (Stomach) Cancer	[43]
Liver Cancer	[44]
Lung Cancer	[45]
Melanoma	[46]
Multiple Myeloma	[47]
Leukemia	[48]
Lymphoma	[49]
Oral Cancer	[50]
Ovarian Cancer	[51]
Pancreatic Cancer	[52]
Prostate Cancer	[10]
Sarcoma	[53]

Table 3
A partial list of signaling pathways linked to reactive oxygen species

Signaling intermediate	Reference
AHR	[98]
AP-1	[99,100]
ATM	[101]
cAMP	[102]
cAMP-dependent PKA	[103]
CDK5	[104]
Chemokine	[70]
c-myc	[99]
CREB	[103]
Cyclins and Cell Cycle Regulation	[105]
Cytokine Network	[66]
DNA Methylation	[106]
DNA Repair Mechanism	[107]
EGF	[108]
eNOS	[109]
ERK	[110]
Fas	[111]
FOXO	[112]
HIF-1α	[113]
HO-1	[114]
IL-10	[115]
iNOS	[109]
Integrin	[116]
Interferon	[117]
JAK/STAT	[118]
JNK	[119]
MAPK	[110]
Mismatch Repair	[120]
mTor	[121]
NAD(P)H quinone oxidoreductase 1	[122]
NF-κB	[123]
Nfr2	[124]
PI3K/Akt	[125]
p38	[126]
p53	[127,128]
PKC	[129]
PPARγ	[130]
PTEN	[131]
PTPs/PTKs	[132]

Signaling intermediate	Reference
Sp1	[133]
TNF	[5]
VEGF	[134]
WNT	[135,136]